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LIFE HISTORY OF *LEUCOCYTOZOON SIMONDI* MATHIS AND LEGER IN NATURAL AND EXPERIMENTAL INFECTIONS AND BLOOD CHANGES PRODUCED IN THE AVIAN HOST[1]

BY A. MURRAY FALLIS, DOUGLAS M. DAVIES, AND MARJORIE A. VICKERS

Abstract

Ducks exposed outdoors in Algonquin Park during the summer became infected with *Leucocytozoon simondi* and many of them died from the infection in June and July when black flies were abundant. The minimum prepatent period was five and a half days. Young parasites were observed in erythrocytes and lymphocytes; mature gametocytes, as shown by exflagellation of microgametocytes, occurred in round and elongated host cells. Asexual development was observed in the spleen, liver, heart, brain, lung, lymphoid tissue, and pancreas. The schizonts were large and contained, at maturity, more than a million merozoites about 1 μ in diameter. Sexual development of the parasites within two species of black flies was completed in three days at summer temperature. Ookinetes were present in the stomach of black flies about four to six hours after the flies had ingested blood containing gametocytes. Developing oocysts were found free among the stomach contents of flies 40–48 hr. after the blood meal. Sporozoites were observed between 60–70 hr. after the flies had ingested gametocytes. Artificial infections were produced in ducks following the injection of macerated black flies that had fed two and one-half to seven days previously on infected ducks. The resulting infections were less severe than those that resulted from natural infections, although the pattern was similar and a minimum prepatent period of eight days was observed. The asexual cycle was completed in fewer than six days although some asynchronicity was apparent. Artificial infections were produced following the injections of blood, spleen, liver, lung, and bone marrow that were taken from ducks at various intervals following their exposure to natural infection. The minimum prepatent period in these infections was eight days and low parasitemias were produced. Gametocytes survived for at least one week in peripheral blood. Ducks developed some resistance following repeated infections but single infections did not protect ducks that were exposed to infection six weeks later. Heavy infections developed in ducks that were splenectomized. The leucocytosis and anemia associated with infections were measured and recorded.

Introduction

Species of *Leucocytozoon* have been recorded from a number of birds in Canada (2) and a high incidence among some of our game birds has been noted (6). Wickware (18) described the species that is found in ducks in Canada and pointed out its pathogenicity. O'Roke (15) made a most valuable contribution when he worked out the general features of the life history of

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L. simondi (=*L. anatis* Wickware). Huff (9) and Wingstrand (19, 20) contributed to our knowledge of schizogony in the genus. The present study of *L. simondi* has been concerned with the stages of development of the parasite and the time intervals between them, the parasitemias produced by natural and artificial infections, and some of the effects of the parasites on the host.

Materials and Methods

The parasites were studied in White Pekin and White Indian Runner ducks. Most of the ducks were one to six weeks old when infected naturally or artificially although infections in some ducks were followed for over a year. Natural infections were acquired by exposure of the birds in open pens in Algonquin Park at the Wildlife Research Station of the Department of Lands and Forests of Ontario.

Black flies were given blood meals by placing them in vials, the open ends of which were placed on areas of infected birds from which the feathers were clipped so that the flies could contact the skin more easily. These flies were maintained for various periods in captivity by feeding them on sucrose. They were then macerated in Tyrode's solution and the resulting suspensions were injected intraperitoneally and intravenously into ducks kept in the laboratory at Toronto away from the black fly area. Saline suspensions of macerated tissues from infected ducks were injected intraperitoneally and subcutaneously into normal ducks.

Tissues from normal and infected ducks were fixed in Zenker–formol solution and sections of these were stained with haematoxylin–eosin–azure. Blood smears were fixed in absolute ethanol and stained with Giemsa. A relative estimate of parasites per bird was obtained by two minute counts of the number of parasites on these blood smears using 10 × ocular and 20 × objective.

Blood samples were transferred from ducks into tubes containing 0.1 cc. of a solution of 4% potassium oxalate plus 6% ammonium oxalate that had been evaporated to dryness. Blood cell volume was determined by centrifuging 0.1–0.4 cc. of the citrated blood in Bauer and Schenk protein centrifuge tubes. Haemoglobin determinations were made by the Sahli method. Red blood cell counts were made on a 1/200 dilution of blood and diluent, and white cell counts were made on a 1/20 dilution of blood and diluent. Blood was drawn from the ducks on alternate days.

Results

Natural Infections

Several hundred ducks have been exposed to natural infections during the past three summers. Smears made from 126 of these birds showed that the minimum period from time of exposure to appearance of parasites in the peripheral blood was five and one-half to six days, although a longer interval elapsed in many instances. This suggested that the prepatent period was probably not much less than six days. The longer intervals may have been

the result of a longer prepatent period or of lighter infections that were not so easily detected in blood smears, or it may be that infection was not acquired on the first day of exposure. The length of the prepatent period was tested therefore, by exposing a series of 14 ducks for 24 hr. and taking blood smears to determine when parasites appeared. The prepatent periods observed in these ducks varied from 6 to 12 days, the average being 7 days.

Ducks became infected naturally from the last week in May until the first week in September. The length of exposure of the ducks before appearance of parasites in the blood varied throughout the summer and appeared to be related to the abundance of black flies in the field (Table I) particularly

TABLE I

RELATION BETWEEN NUMBER OF BLACK FLIES AND INFECTION IN DIFFERENT MONTHS (1949)

Month	Abundance of flies	Time in days from exposure of ducks to appearance of parasites			Number of ducks		
		Max.	Min.	Av.	Exposed	Infected	Died
May	Scarce	22	19	21	15	15	0
June	Abundant	14	6	7.9	32	32	12
July	Abundant	14	6	7.4	24	24	12
August	Scarce	32	7	21	25	7	4

to that of *Simulium venustum* Say which species was shown to be a vector by O'Roke (15). It will be observed also (Table I) that there was a higher incidence of infection and greater mortality among infected birds in June and July when the black flies were abundant (4). After the first week in June there were always infected ducks in the area and this no doubt, intensified the rate of transmission. In some years infection was obviously present in the field in May, as ducks exposed on May 30 became infected and showed parasites in the blood in seven days and some died two days later. The prevalence of the infection is indicated also by the results of an experiment in which 12 ducks were exposed in an area where there had been infected ducks for six weeks previously, while three ducks were exposed at the same time three miles away. Ducks in both areas had parasites in their peripheral blood six days later. None of the birds in the outlying area died from the infection but all of the ducks died in the area where infection was known to exist previously. Infection has been observed in several species of wild ducks from widely separated areas in Ontario but the incidence has not been determined.

The parasitemias following natural infections showed considerable variation (Fig. 1). They reached a peak usually four to eight days after the parasites were observed in peripheral blood, the average time being six days in one series of 15 infections (Fig. 2). This peak was reached in fewer than 10 days regardless of the number of days' exposure of the ducks. The parasitemia usually declined rapidly although it remained high for a longer period in the birds that were exposed to repeated infections (Fig. 2) as compared to those exposed

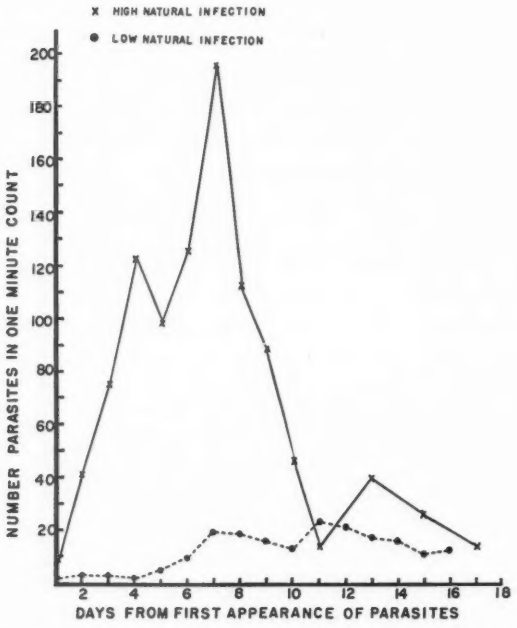


FIG. 1. High and low parasitemias in ducks following natural infections.

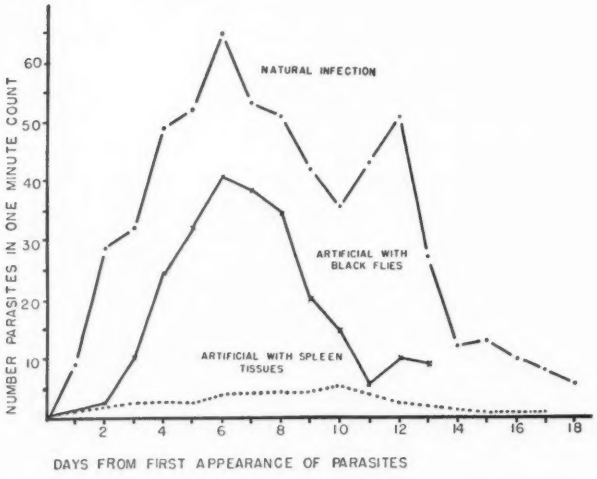


FIG. 2. Average parasitemias in 15 ducks following natural infections, in 15 ducks following artificial infections produced by injections of infected black flies, and in 15 ducks following artificial infections produced by injections of infected tissues.

to a single artificial infection. The parasitemias and the number of days from exposure to appearance of parasites in the blood was followed in 12 one-week-old ducks that were subjected to possible natural infections as follows: two ducks for one day, three ducks for two days, three ducks for four days, and four ducks for six days. It will be noticed (Table II) that the prepatent

TABLE II

PARASITEMIAS IN DUCKS EXPOSED TO INFECTION FOR VARIOUS PERIODS

	1 day exposure		2 day exposure			4 day exposure			6 day exposure			
	Duck number											
	222	223	225	226	228	230	231	232	234	235	236	237
	Days from exposure to infection											
	12	7	11	10	7	14	7	14	8	12	13	7
	Parasites in 1 min. counts											
June 10		+			+		13					4
June 11		33			3		51		+			19
June 12		124			37		68		11			67
June 13		48		+	47		19		24			16
June 14		57	2	2	58		35		28			47
June 15	+	16	9	4	160		22		28	+		114
June 16	+	16	6	19	117		20		15	1	+	47
June 17	15	15	22	46	41	+	27	+	13	5	1	49
June 18	38	54	13	92	54	+	64	+	18	9	13	36
June 19	82	45	18	84	44	2	36	+	60	+	16	41
June 20	57		28	81	154	4		5	87	20	28	
June 21	125		35	59		19		30	79	94	59	
June 22	61		37	41		30		121	74		21	
June 23	43		47	34		60		95	37	191	5	
June 24	23					26		35			3	
June 25	15					21		56			2	
June 26						12		7			1	
June 27						4		16				
June 28						3		33				
June 29								26				

period was longer in some ducks than in others and the difference cannot be explained in most instances by assuming that the infections were acquired at different times. No correlation was observed between the height of the parasitemia and the number of days exposure, or the time required to reach it.

Infections persisted at low chronic levels from one summer to the next. There were some fluctuations in the levels of the parasitemias in eight ducks that were observed during the winter but the parasitemias were always low compared to those observed following re-exposure to natural infections the next summer. Moreover two of the eight ducks carrying chronic infections

died with heavy parasitemias nine days after re-exposure to natural infections at the beginning of June, indicating that the chronic infections did not protect them against reinfection.

The typical forms of the gametocytes of this parasite, as seen in peripheral blood, have been described as elongated, the host cell attenuated with its nucleus drawn out and pushed to one side of the cell. It is difficult to decide the type of such host cells. In many birds from which blood smears were made daily, commencing six days after exposure, it was possible, at the beginning of the infection, to find large as well as small parasites in host cells that retained their round appearance. Small forms, some only 1 μ in diameter, were found in erythrocytes, confirming the observations of Hartman (8). Other small parasites have been observed in cells of the lymphocytic series. Thus, more than one type of cell is invaded which may account for the diverse reports of the types of parasitized host cells (9). The presence of many small parasites and large round forms was more noticeable in ducks that were exposed to continuous infection and had high parasitemias. These small and large round parasites were usually more numerous at the beginning of the infection, especially in ducks exposed for a single day. The relative number of large parasites in round and elongate host cells and the length of time one type predominated varied in different ducks (Fig. 3). The significance of the two types is not understood although evidence from blood transfusions, presented later, suggested that they were in two types of cells. This view is supported also by examination of the centrifuged layers of blood in which there appeared a greater tendency for the elongate cells to remain in the top, and the round cells in the bottom layer of blood. This might be due of course to other causes, such as changes in specific gravity in the parasitized cells.

Some of the round as well as the elongate forms were mature as shown by their ability to exflagellate *in vitro*. It was apparent also that some of the gametocytes mature rapidly, as exflagellation was observed seven days after exposure and one day after the first parasites were detected in the blood. It is possible that young parasites were in the blood prior to six days after infection and these had reached maturity by the seventh day rather than that the young parasites observed on the sixth day were mature a day later. The rate of maturation of parasites was followed also by feeding black flies daily on infected ducks commencing on the day the parasites were detected and then dissecting the stomachs of the black flies 12 hr. after the experimental feedings and counting the number of ookinetes. This confirmed the results of the *in vitro* studies on exflagellation but, owing to difficulties in counting, we obtained no significant quantitative data on the relation between the number and types of gametocytes in the blood and the number of ookinetes. It appeared most probable also that some of the elongate parasites were no longer viable as they were observed within the blood cells in the stomachs of black flies 74 hr. after the flies had fed on infected ducks. There would appear to be differences in the rate of maturity of the gametocytes and/or asynchronicity in their production, as shown by the size of the parasites in the peripheral

blood of a duck that developed an infection in more than 75% of the blood cells as a result of exposure in the field for one-half hour. Ten and one-half days after exposure the duck had parasites ranging in size from 1 μ to 12 μ in the peripheral blood.

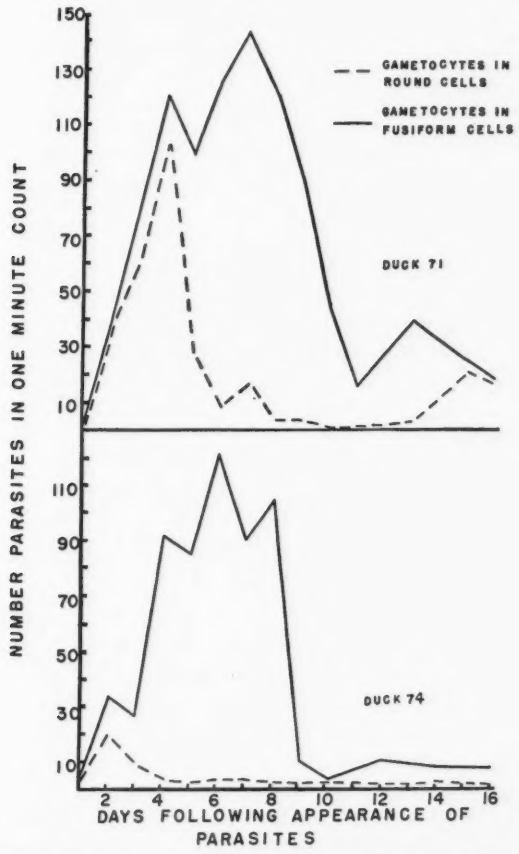


FIG. 3. Number of parasites observed in round and elongate host cells of two ducks on successive days following the appearance of parasites in the blood.

Development in Black Flies

Development of the parasites was followed after the ingestion of gametocytes in the blood meal of various species of black flies that were identified by one of us (D.M.D.). Black flies were derived from two sources for this part of the investigation: (1) those that emerged and had landed on humans, (2) those that were caught in a fine mesh (30 mesh per in.) cage as they emerged from a stream. It was only after much patience and perseverance that a few

of the latter could be induced to feed on the ducks, consequently most of the observations reported below were made on flies that were caught as they landed on humans.

More flies were induced to feed on ducks if the vial was darkened except for a small portion close to the bird's skin. More flies fed at certain times and on certain days than others, as might be expected from the results of Davies (3) on the effect of environmental factors on the activity of black flies.

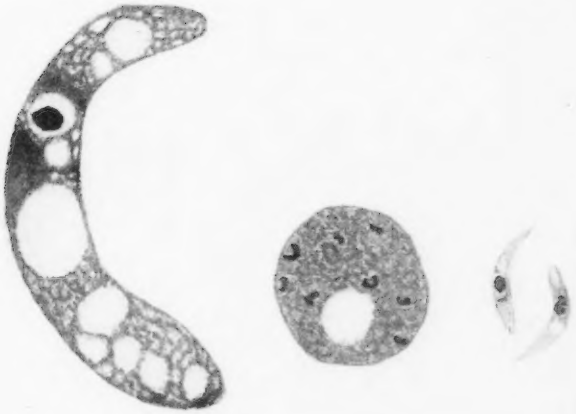
The flies, after feeding, were maintained in tubes and fed on sucrose (5). Survival of the flies was improved by giving them a partial rather than a full blood meal, consequently we tried to remove them from the ducks before they had taken their maximum amount of blood. This had some disadvantages as all flies did not feed at the same rate so that some were probably removed before they had taken any blood.

The black flies were used in various ways following these experimental feedings. Some were identified, the alimentary tract and salivary glands removed by dissection under saline on a slide and observed immediately under high power. These and other similar preparations were dried on slides, stained with Giemsa and examined under high power and oil immersion. Other groups of these presumably infected flies were emulsified in Tyrode's solution on various days after they had fed on infected ducks. These preparations were then injected intraperitoneally and intravenously into noninfected ducks. In some instances the alimentary tracts and salivary glands were dissected from the flies, macerated, and injected separately into ducks. As a control to these procedures, smear preparations were made from several hundred flies that were not known to have fed on infected ducks and similar flies were macerated and injected into normal ducks. These studies have shown that exflagellation of the microgametocytes may occur in the stomach of the black fly within one or two minutes after their ingestion. Zygotes were common two hours after the black fly had ingested infective gametocytes. The zygotes may change in four to six hours into ookinetes (Fig. 4) although the rate of change was apparently subject to some variation. Ookinetes were still present in the stomachs of some black flies 74 hr. after the ingestion of the parasites. It is suspected that these latter ookinetes were dead, although they were in species of flies that were suitable hosts as shown in the experiments reported below. Development to the ookinete stage was obtained in the following species: *Simulium venustum* Say, *S. vittatum* Zett., *S. decorum* Walker, *S. tuberosum* Lund., *S. parnassum* Mall., and *Prosimulium hirtipes* (Fries). Development of the parasites to the infective stage in black flies took place in *S. venustum* and *S. parnassum* as shown by the injections of suspensions of these flies into ducks.

O'Roke (15) reported the finding of oocysts on the outer wall of the stomachs of black flies 22 hr. after the insects had been removed from ducks.* We have made preparations of the salivary glands and stomachs of 160 flies that were

* *Kartmann (Pacific Sci. 3 : 127-132. 1949), in his study of Haemoproteus columbae in pigeons, has found and illustrated oocysts on the mid- and hindgut of hippoboscid flies.*

PLATE I



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Stages of development of *L. simondi* as observed in smears prepared from black flies at various intervals after they had fed on infected ducks. FIG. 4. Ookinete at 12 hr. FIG. 5. Developing oocyst at 40 to 48 hr. FIG. 6. Sporozoites at 72 hr. Giemsa stain.

not fed experimentally as well as from more than 400 flies that had been fed 2 to 12 days previously on infected ducks. We have been unable to demonstrate oocysts on the outer wall of the stomach of these flies. On the other hand, 40 to 60 hr. after the fly had fed, some ookinetes were assuming a round shape and the chromatin in them was divided. Moreover in smears made from flies 40 to 105 hr. and particularly 40 to 48 hr., after they had fed on infected ducks we have seen structures that we consider to be developing oocysts (Fig. 5) among the stomach contents of the fly. The chromatin in these developing oocysts was divided into a number of separate entities and appeared as if it were likely to undergo further subdivisions. There were too many of these oocysts free among the stomach contents to assume that they had been detached from the stomach wall in making the preparations. Twenty-two of these developing oocysts measured 6.4 to 9.6 μ in diameter, the average being 8.2 μ.

Structures that may be almost mature oocysts were observed in the stomach of a fly 67 hr. after it was fed on an infected duck. These measured 10 μ to 13 μ in diameter and appeared to be dividing into structures that resembled sporozoites. Similar sporozoites (Fig. 6) have been observed in stained smears, chiefly among the stomach contents and to a lesser degree, close to the salivary glands of black flies 60–70 hr. after they had fed on infected ducks. These sporozoites measured 5 to 10 μ in length and were more sharply pointed at one end than the other, with the nucleus located nearer the blunt end. Vacuoles occurred in some of them. Sporozoites have not been observed streaming out of the cut ends of the salivary glands.

Artificial Infections Using Black Flies

Evidence of the rate of development of the parasites in the black flies was obtained also by macerating them in Tyrode's solution at intervals following their feeding on infected ducks and injecting the suspensions into parasite-free ducks. In some instances a suspension of salivary glands was injected into one duck and a suspension of the remainder of the same flies into another duck. Infections were produced in 35 ducks by suspensions containing 4 to 44 flies that had fed two and one-half to seven days previously on infected ducks. The inoculum was prepared from the salivary glands, the alimentary tracts, and from entire flies. The prepatent period varied from 8 to 14 days and averaged 12 days. The average height of the parasitemias in these artificial infections (Fig. 2) was lower than that observed for the natural infections although the pattern was similar. None of the ducks died as a result of these infections in contrast to the 50% to 100% mortality in some groups of ducks that were infected naturally. The height of the parasitemia and the length of the prepatent period was tested in relation to the number of sporozoites the birds received, by injecting ducks with different quantities of a saline suspension of macerated black flies (Table III). There was a slight indication that the prepatent period was shorter in the ducks that received the larger amounts of the suspensions although the height of the parasitemia was not necessarily higher. Transmission of the parasite through the "bites" of

TABLE III

COMPARISON OF THE PREPATENT PERIOD IN DUCKS INFECTED ARTIFICIALLY WITH DIFFERENT QUANTITIES OF SUSPENSIONS OF INFECTED BLACK FLIES

Experiment	Duck No.	Relative size of dose	Prepatent period, days
I	269	4	13
	270	2	14
	271	1	14
II	324	2	12
	325	1	14
	327	1	11
III	355	2	9
	356	1	13
IV	358	2	11
	359	1	11
V	368	3	9
	369	1	10
VI	575	2	16
	576	1	16

infected black flies rather than by ingestion of them was demonstrated in a duck that had its beak taped shut during its exposure to infection. Moreover no infection developed in eight ducks that were fed 3 to 34 black flies that had received blood meals from infected ducks two and one-half to seven days previously nor in four ducks that were fed 50 to 150 black flies of unknown history.

Asexual Development and Artificial Infections

It is apparent from the natural infections that a cycle of schizogony is completed in less than a week as parasites were present in the blood of many ducks six days after infection. There are several reports, for different species of this genus, of schizonts in the tissues of the host but the valuable contributions of Huff (9) and Wingstrand (19, 20) give more detailed descriptions than any presented previously. Huff found two types of schizonts. One type was smaller than the other and was found in liver cells. The larger type, which he called a megaloschizont, was found in heart, spleen, intestine, and liver. We have found schizonts, corresponding to the megaloschizonts of Huff, in various stages of development in spleen, liver, lung, heart, pancreas, brain, and lymphoid tissues but none were seen within liver cells (Fig. 7). The schizonts were most numerous in the spleen especially in those ducks in which more than one cycle of schizogony may have occurred before they were sacrificed. Schizonts were scarce in most tissues except in heavy infections. It appears probable that the schizonts developed in macrophages as Huff and Wingstrand reported. Ducks were exposed to natural infection for 36 hr.

or less and then sacrificed at intervals thereafter and their tissues fixed, sectioned, and examined. Schizonts in various stages of development were observed in the following tissues at intervals after infection: spleen, four to five days; spleen, five to six days; spleen, six to eight days; spleen, seven to nine days; heart and spleen, 10 to 11 days; spleen, liver, lymphoid tissue, brain, heart, lung, 10½ days. The schizonts, seen in tissues five to eight days after infection, were small (Fig. 8); those in the spleen seven to nine days after infection were larger (Fig. 9); and some of those in the spleen of one duck 10½ days after infection were almost mature, although smaller schizonts were found in other tissues (Fig. 10). It was observed that within a single tissue, the spleen, the schizonts were not all at the same stage of development, and even within the same schizont there were cells that were considered to be mature merozoites and others that were presumably immature. These mature merozoites were similar to those that were seen in other mature schizonts (Fig. 11) the age of which was unknown. This indicated that development within the schizont was not necessarily uniform. Twenty of these large schizonts had diameters of 115 μ to 168 μ with an average of 138 μ. The merozoites measured about 1 μ or less. Obviously then each of these large schizonts contained more than a million merozoites. A relatively small number of schizonts could therefore soon flood the blood stream with parasites, assuming that many of these merozoites invade the blood cells and become gametocytes. Consequently if these schizonts were located in various tissues there may have been few of them in any one tissue and they would be difficult to find.

Asexual development was studied also by removing tissues at intervals from infected ducks and transferring them to normal ducks. In the first experiments tissues were taken from ducks that were exposed continually in the field until parasites appeared in the blood. Consequently they may have been infected on successive days during exposure and if so the tissues could contain schizonts in different stages of development. These ducks were sacrificed 1 to 13 days after the parasites appeared and emulsified tissues from them were injected intraperitoneally into 34 other ducks, 20 of which became infected. Seventeen of these positive ducks received spleen, one brain, one lung, and one liver tissue respectively. These tissues had been removed from ducks one to five days after their last possible natural infection. No infection occurred in 14 ducks, 10 of which received spleen, one brain, one liver and two lymphoid tissue respectively. With two exceptions, the tissues, that were taken from ducks 6 to 13 days after their last possible natural infection did not produce infections when injected into other ducks.

These experiments, although indicating that infections could be transmitted by transferring various tissues that must have contained the asexual stages, gave no indication of their rate of development. Additional experiments were conducted therefore, in which ducks were exposed to natural infections for known periods of 48 hr. or less, then removed to the city where there were no black flies. They were sacrificed at intervals following exposure and the

spleen and other tissues were emulsified and injected into other ducks. This had certain disadvantages as we did not know whether all the ducks became infected while exposed, and if so, whether at the beginning or end of their exposure, or whether they were infected more than once. Daily blood smears were made from each of the injected ducks beginning 7 days after their injections and continuing for 10 days.

Experiment I:—Fifteen ducks were exposed for 36 hr. to possible natural infection. At intervals of 3 to 17 days after exposure (Table IV) one, two, or three of these ducks were killed and portions of their spleens were emulsified in saline. The resulting suspensions were pooled and injected intraperitoneally into two parasite-free ducks. Infections were produced in ducks injected with spleen tissues containing parasites that arose from natural infections acquired between three to five days, four to six days, and seven to nine days previously. No infections developed from injections of spleens taken from ducks that might have been infected by exposures 6 to 8 days, 12 to 13 days, 14 to 15 days, and 16 to 17 days previously. Nevertheless some of these ducks contained developing parasites as immature schizonts were seen in ducks, six to eight days (Fig. 8) and seven to nine days (Fig. 9) respectively after natural infection. Moreover gametocytes were observed in the blood of the ducks that were sacrificed 7 to 17 days after exposure. This suggests that the ducks sacrificed before seven days after exposure were probably positive also.

Experiment II:—The procedure was similar to that in the first experiment except that 12 ducks were exposed to infection for 24 hr. only, and other tissues in addition to spleen were used for injections. The results of the experiment are summarized in Table IV. It will be observed that infections resulted from the injections of spleen tissues in which the parasites had been developing for 48 to 77 hr., four to five days, and five to six days and from the injections of liver tissues in which they had been developing for four to five days and six to seven days respectively.

Experiment III:—In this experiment the ducks were exposed to infection for three hours only and blood, bone marrow, and spleen tissues from them were injected into other ducks (Table IV). Infections were produced as a result of

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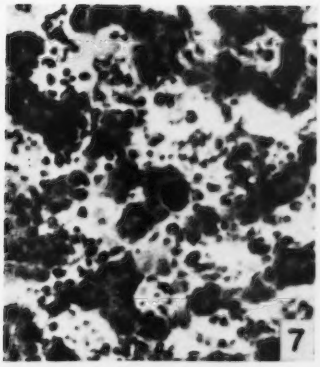
FIG. 7. Schizont in liver of duck 10½ days after infection. The schizont is partially developed and is apparently outside a liver cell. Liver degeneration is also apparent.

FIG. 8. Young schizont observed in spleen of a duck that was infected naturally between six and eight days previously. Similar schizont was seen in spleen five to six days after infection.

FIG. 9. Developing schizont in spleen of duck that was infected naturally between seven and nine days previously.

FIG. 10. Developing schizont in lymphoid tissue of duck that was infected naturally 10½ days previously. Schizonts that were almost mature were seen in the spleen of this same duck.

FIG. 11. Schizont containing merozoites in spleen. Similar merozoites were seen in some schizonts that were present in the spleen of a duck 10½ days after it was infected naturally.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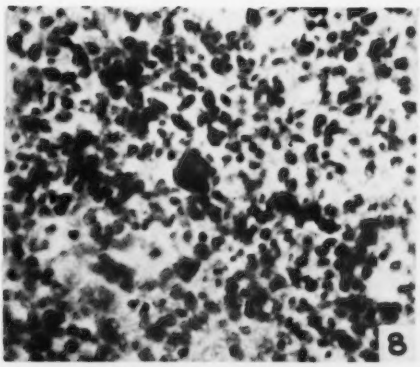
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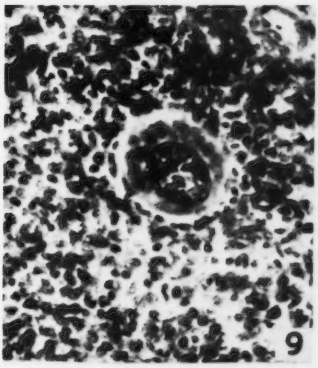
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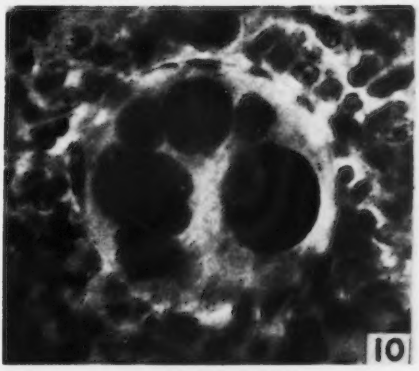
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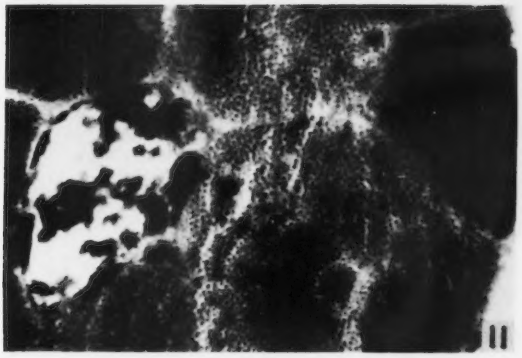
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TABLE IV

ARTIFICIAL INFECTIONS BY INJECTIONS OF TISSUES REMOVED FROM DUCKS AT VARIOUS TIMES FOLLOWING THEIR EXPOSURE TO NATURAL INFECTION

Duck No.	Tissue injected	Possible age parasites in tissue injected	Prepatent period (days)	Remarks
Experiment I				
221	Spleen	3–5 days	15	Injected with spleen from same ducks
227	Spleen	3–5 days	15	
264	Spleen	4–6 days	9	Injected with spleen from same ducks
265	Spleen	4–6 days	—	
266	Spleen	6–8 days	—	Injected with spleen from same ducks. Young megaloschizont in spleen of one donor duck
267	Spleen	6–8 days	—	
348	Spleen	7–9 days	—	Injected with spleens from same ducks. Young megaloschizont in spleen of one donor duck
349	Spleen	7–9 days	14	
No infection was produced in three ducks injected with spleen tissues that were removed from ducks 12–13, 14–15, and 16–17 days after exposure and that had parasites in blood.				
Experiment II				
519	Spleen	48–72 hr.	14	Injected with tissues from same ducks
501	Liver	48–72 hr.	—	
502	Intestine	48–72 hr.	—	
520	Spleen	4–5 days	10	Injected with tissues from same ducks
521	Liver	4–5 days	14	
522	Intestine	4–5 days	—	
523	Spleen	5–6 days	9	Injected with tissues from same ducks. Small megaloschizont in one donor duck
524	Liver	5–6 days	—	
525	Intestine	5–6 days	—	
526	Spleen	6–7 days	Died	Injected with tissues from same ducks
527	Liver	6–7 days	14	
528	Intestine	6–7 days	—	
Negative results were obtained using similar tissues removed from ducks 8–9, 10–11, and 15–16 days after their exposure, although 6–11 days after exposure all donor ducks had parasites in blood and young megaloschizonts were observed in donor ducks 8–9 and 10–11 days after exposure.				
Experiment III				
644	Blood	70–73 hr.	8	Injected with tissues from same ducks
645	Spleen	70–73 hr.	—	
646	Bone marrow	70–73 hr	8	
654	Blood	106–109 hr.	16	Injected with tissues from same ducks
655	Spleen	106–109 hr.	—	
656	Bone marrow	106–109 hr.	—	
Negative results were obtained using similar tissues removed from ducks 82–85, 97–100, 120–123, 132–135, and 145–148 hr. respectively after exposure.				

injections of blood and bone marrow removed from ducks three days after exposure and by blood removed from ducks four and one-half days after exposure.

In a fourth experiment no infections resulted from injections of spleen tissues removed from ducks two, three, and six days respectively after exposure for four hours.

The prepatent period in these artificial infections varied from 8 to 21 days, the average being 13.5 days. The minimum prepatent period was the same as that obtained in the artificial infections with black flies but was three days more than that reported by Chernin and Sadun (1) following tissue inoculations. The average parasitemia in these infections was low (Fig. 2), not unlike the occasional low parasitemia that was observed following a natural infection (Fig. 1). Small parasites were not observed in these artificial infections. Moreover most of the parasites were in elongate rather than in round host cells. None of the ducks died as a result of the infections.

It is apparent from these experiments that infective stages of the parasites were present in the blood in three, and in four and one-half days after natural exposure; in the bone marrow in three days; in the liver in four to seven days; and in the spleen in two to six and in seven to nine days after exposure. This could mean that an asexual cycle was completed in three days; or that the parasites were developing in certain cells transferred by the injections, and growth and development continued in the new host. If the first asexual generations produced only another generation of schizonts it would explain our success in transferring infections at intervals of three and four days, before parasites were detected in the blood. If such were the case the asexual generations would build up the infection and the transfers made at three days would be expected to produce higher parasitemias than those made at five to six days. However, this did not occur. Moreover there was no marked difference in the length of the prepatent period in the artificial infection produced by injections of tissues as compared with that produced by injections of black flies. No stages of schizogony have been detected in the peripheral blood although such have been reported by various authors (7, 8, 11, 14). Moreover the transfer of infection with blood taken from ducks three and four and one-half days after infection shows that asexual parasites were in it. It is probable that these asexual stages get into certain lymphocytic cells that may circulate for a time and are finally caught up in various tissues and grow into macrophages, while at the same time the asexual development of the parasites proceeds within them. The low concentration of the developing asexual parasites in any one tissue, and therefore their absence in the inoculum may explain some of the failures to transmit infections artificially.

Longevity of Gametocytes

The survival time of gametocytes in the peripheral circulation was followed in ducks that were injected intravenously with gametocytes in blood from infected ducks (Table V). It will be observed that parasites were found in the blood of some of the injected ducks for several days and after five weeks

TABLE V

SURVIVAL OF GAMETOCYTES WHEN INOCULATED INTO OTHER DUCKS

Duck No.	Date donor positive	Donor exposed to infection until	Date blood used for injection	No. gametocytes per 1000 R.B.C.'S	Amount blood injected (cc.)	Results of examination of blood of inoculated ducks
708	Aug. 15	Aug. 15	Aug. 18	26	1	Parasites in round and elongate cells Aug. 18–23; none observed Aug. 24–26; more numerous in round and elongate cells Aug. 27
714	Aug. 21	Aug. 21	Aug. 24	46	0.3	Parasites in round and elongate cells Aug. 25–30; negative Aug. 31; positive in elongate cells Oct. 2
715	Aug. 24	Aug. 24	Aug. 24	12	0.3	Positive until Aug. 29, no parasites observed Aug. 31–Sept. 4
718	Sept. 5	Sept. 1	Sept. 8	9	1.2	Parasites in round and elongate cells until Sept. 14; negative Sept. 20; positive Sept. 26
719	Sept. 5	Sept. 1	Sept. 8	32	0.8	Parasites in round and elongate cells until Sept. 19

in No. 714. It would appear that the parasites may survive for at least six days and it is probable that some remain in the circulation for longer periods. Previous experiments showed that an asexual cycle may be completed in fewer than six days and after seven days mature gametocytes may be found in the blood. Artificial transmission by blood was also demonstrated. It follows, therefore, that parasites found in ducks eight days after an inoculation of blood containing gametocytes might be either those injected or those that had arisen from a new generation. It is most probable, however, that the parasites found five weeks after the original injection into duck No. 714 were the result of a new generation. It is possible also that new infections were produced in ducks Nos. 708 and 718. Parasites were observed for five to six days following inoculations into these ducks, after which they were not observed for a few days but subsequently became more abundant and were present 9 and 18 days respectively after the ducks had been injected. Large parasites were observed in round and elongate host cells at the time of the transfusions and for several days thereafter, which suggests that round host cells may not change into the elongate type when they contain large parasites. This may be a further indication that they are in two types of host cells, one of which elongates soon after the parasite enters it while the other remains round.

Blood Changes Caused by Infection

The pathogenicity of this and related species of parasites has been discussed by Wickware (18), O'Roke (15), Skidmore (16), Knuth and Magdeburg (11), Stephan (17), and others. The evident anemia caused by the infection prompted us to make a quantitative study of some of the blood changes that were apparent during the course of the infection.

Changes in the blood were followed in 25 ducks that were infected naturally by exposure of one to several days. Determinations of haemoglobin, blood cell volume, and number of red and white blood cells were made on alternate days over a period of 2 to 17 days depending on the survival of the duck. The results were compared with a series of 27 determinations from 11 non-infected ducks of similar age. The values for the normal ducks (Table VI) were similar to those obtained by Magath and Higgins (13) on the blood of mallard ducks, although we obtained slightly lower average values for the amount of haemoglobin and the number of white blood cells.

TABLE VI

SUMMARY OF BLOOD VALUES BASED ON 27 DETERMINATIONS FROM 11 NONINFECTED DUCKS

	Values		
	Lowest	Highest	Average
Haemoglobin, gm. per 100 cc.	10.25	12.7	12.1
Erythrocytes, millions per cu. mm.	2.78	3.64	3.5
Red cell volume, % of total vol.	34	51	43
Leucocytes, thousands per cu. mm.	10	18	12

The pattern of the blood changes was similar in all the infected ducks although there were differences in the absolute values. This might be expected as the ducks were exposed to infection for different periods and some developed heavier infections than others. The average as well as the extreme values observed in the ducks tested on different days after infection are shown in Figs. 12 and 13. It is apparent that there was an increase in the number of leucocytes just before or at the time the parasites were detected in the peripheral blood. They became more numerous until about four to six days after the parasites were first observed, following which they decreased to normal about three weeks after the patent infection began. The number of red blood cells, the blood cell volume, and the amount of haemoglobin began to decrease as the infection appeared. The decrease continued for about one week after infection appeared and the values returned slowly to normal in approximately three weeks. In some infected ducks blood cell volume was 20% of normal and the number of red blood cells and the amount of haemoglobin were

correspondingly low. In addition to the anemia other noticeably pathological features were the thin vascular walls, the liver degeneration and hypertrophy, and enlargement of the spleen from 10 to 20 times normal size. The spleens of several ducks showed enlargement by the time the parasites were in the peripheral blood although the maximum size was not attained until later.

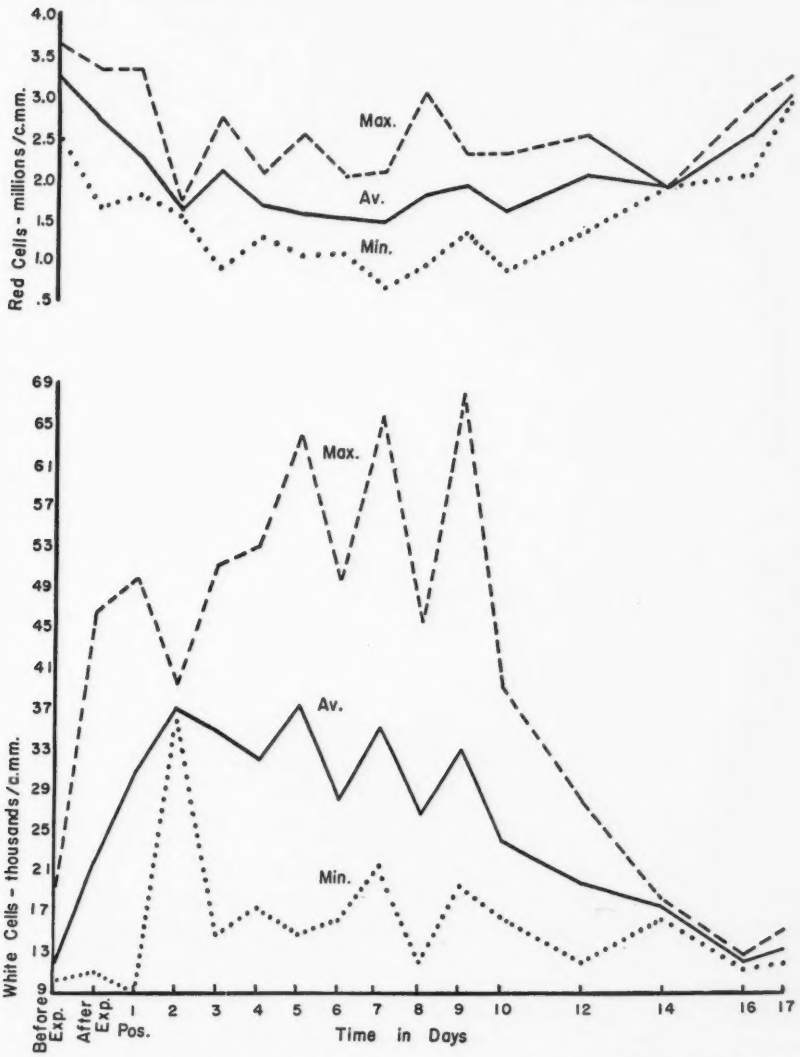

FIG. 12. The number of red and white blood cells in the peripheral blood of ducks before and after natural infections with *L. simondi*.

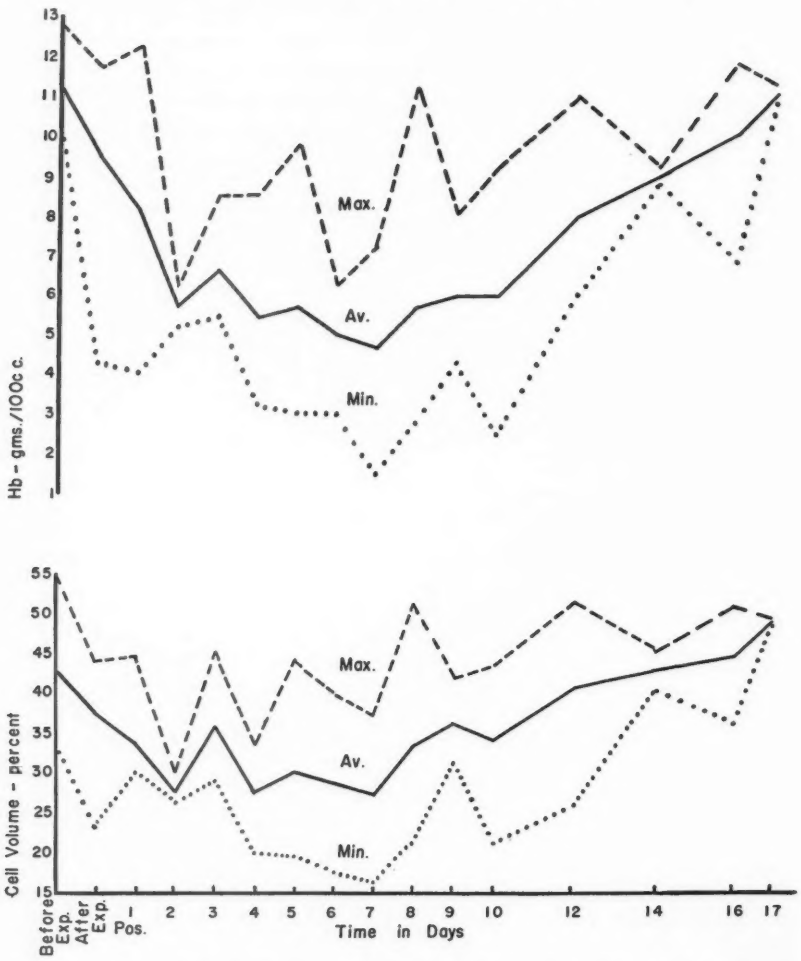

FIG. 13. The red blood cell volume and the haemoglobin values of the peripheral blood of ducks before and after natural infections with *L. simondi*.

Resistance to Infection

A few experiments were arranged to test the resistance of ducks of similar age to reinfection. Some ducks that were exposed continuously for many weeks showed low levels of parasitemia following the initial peaks (Fig. 14). They were presumably reinfected during this time, as new groups of uninfected ducks of similar age developed heavy infections (Fig. 14) when exposed beside the former. The difference in the level of parasitemia suggested that the ducks exposed continuously had become resistant. It was noticed, however,

that ducks with a low grade chronic infection developed a high parasitemia and some died following exposure to infection a year later, therefore a low grade chronic infection did not necessarily render them immune. A further experiment was set up to determine whether ducks were resistant as a result of single

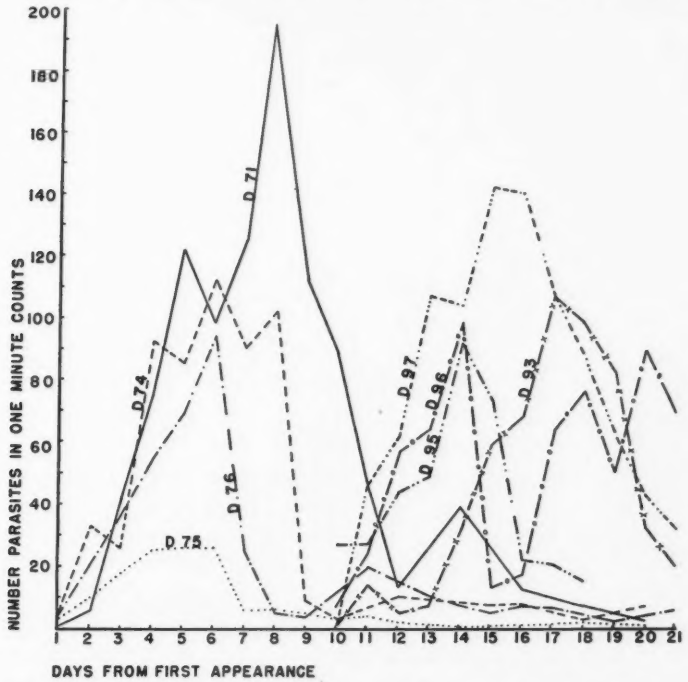

FIG. 14. Parasitemias observed in two groups of ducks that were exposed continually to infection, but one group was exposed 10 days later than the other.

infections. The resistance was measured by comparing the parasitemias in (1) four ducks exposed continuously to natural infections, (2) four ducks exposed to natural infection for the first time, (3) three ducks exposed to infection six weeks after a previous natural infection, (4) four ducks exposed to natural infection six weeks after (*a*) two of them had been infected artificially by injecting infective black flies and after (*b*) two of them had been infected artificially by injections of tissues containing asexual stages. It was apparent (Fig. 15) that single natural or artificial infections did not induce a resistance in ducks when they were exposed to infection six weeks later although those ducks exposed to continuous infection were resistant.

The presence of a number of schizonts in the spleen and the possible importance of this organ in the defence mechanisms of the host suggested an experiment to determine the effect of its removal on the parasitemias. The course of the parasitemias was observed in four splenectomized ducks and

compared with those in five ducks that were not splenectomized. The ducks were exposed to natural infection for five days commencing seven days after the splenectomies. Two of the splenectomized birds died five and seven days

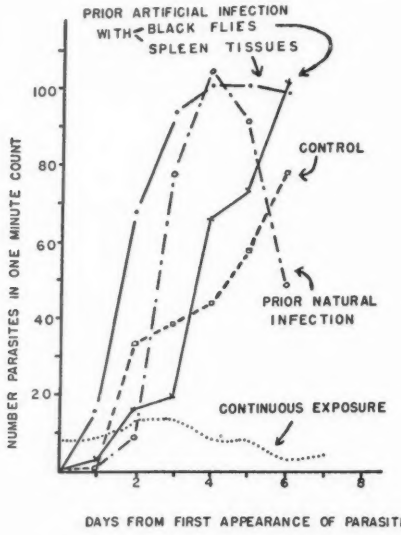

Fig. 15. Average parasitemias observed in ducks, exposed to infection for the first time, exposed to continuous infection, and reinfected six weeks after natural or artificial infection.

respectively after infection appeared in the blood, and one died before infection appeared. Two of the control birds died four days after infection appeared. Parasites were observed in the splenectomized ducks 10, 10, and 14 days and in the control birds 10, 10, 10, 14, and 18 days respectively after their first exposure. No significant differences were noted in the peak parasitemias in the two groups nor in the intervals between the appearance of the parasites and the peak parasitemias. It would appear therefore, that the schizogony that takes place in tissues other than the spleen was sufficient to produce parasitemias equivalent to those that occurred in ducks that were not splenectomized.

In another experiment the spleens were removed from three of seven ducks which had been showing parasites in their blood from one to several days before splenectomy. The parasitemias on the day preceding and for nine days following the splenectomies are shown in Table VII. It will be noted that the parasitemias remained at higher levels in those ducks that were splenectomized which suggested that the spleen was important in the defensive mechanisms of the host. This could account for the larger number of schizonts that have been seen, following the initial cycles of schizogony, in the spleen as compared with other tissues.

TABLE VII

COMPARISON OF PARASITEMIAS IN DUCKS SPLENECTOMIZED AFTER APPEARANCE OF PARASITES WITH THOSE NOT SPLENECTOMIZED

	Number of parasites observed in 2 min. counts							
	Splenectomized ducks			Nonsplenectomized ducks				
	Duck No.							
	339	355	403	227	232	337	404	406
Sept. 6	105	56	40	2	41	35	9	2
Sept. 7	86	41	106	7	36	39	26	4
Sept. 8	90	45	102	—	59	64	19	9
Sept. 9	106	40	76	32	129	89	43	34
Sept. 10	159	58	73	58	111	55	83	52
Sept. 11	167	62	117	80	146	42	79	85
Sept. 12	150	80	195	55	41	31	142	88
Sept. 13	132	105	269	50	12	38	114	66
Sept. 14	19	130	283	9	4	20	158	32
Sept. 15	32	166	203	6	1	27	60	12

Discussion

During the course of this investigation several hundred ducks were infected naturally or artificially with *L. simondi*. The rapidity with which they became infected when first exposed in the spring of some years suggested that a high percentage of the flies were infected and/or that a large number of them fed on ducks. The latter was evident but in spite of dissections and injections of several hundred flies we have been unable to establish the incidence of parasites in them. The rapid rate of infection raises also the question of reservoir hosts. There were a few wild ducks in the vicinity but no domestic ducks. The ease with which the ducks acquired infection in the field compared to the small population of wild ducks suggested that some other bird may be a reservoir host for this species of *Leucocytozoon*. Infection with *Leucocytozoon* is known to occur in several other species of birds in the locality but we have limited data regarding specificity.

The apparent difference in the length of the prepatent period was probably related to the number of parasites present and asynchronicity in schizogony. Small parasites were noticed in heavy infections but they were probably overlooked in light infections so that the presence of large parasites was the first indication of infection. The presence of mature parasites in peripheral blood seven days after infection suggests that the prepatent period may, in some instances, be even less than five and one-half days.

The sporogonic cycle within at least two species of black flies occurred rapidly, as shown by the stages of development found in them when they were dissected at intervals following infection, and by the infections that resulted from injections of them into normal ducks. We have been unable to demonstrate infective stages in flies until two and a half days after the flies

were infected. Neither have we succeeded in demonstrating oocysts on the outer wall of the stomachs of the black flies, but they, as well as sporozoites were found among the stomach contents of infected black flies. Some sporozoites were found in the vicinity of the salivary glands in the stained preparations but none were seen streaming out from the cut ends of salivary glands. It seems most probable, therefore, that oocyst development does not necessarily take place on the outer wall of the stomach of the fly. If penetration of the stomach walls and encystment were always necessary it is improbable that development from zygote to sporozoites would occur in 72 hr. or less. Whether the sporozoites escape from the stomach or salivary glands of an infected fly into the blood stream of the duck has not been determined as infections were produced with material derived from both sources. The greater abundance of sporozoites in the stomach contents than in the vicinity of the salivary glands suggested that they do not necessarily go to the latter, although Johnson *et al.* (10) have a photograph showing a sporozoite of *L. smithi* that appears to be in the salivary gland. It seems to us, however, that more sporozoites should have been found in the salivary glands if the glands are the usual site.

It seems probable that there is a single asexual generation prior to the appearance of gametocytes in the blood but the time required for it is not necessarily the same for all schizonts as indicated by the asynchronicity of development in ducks infected at one time and the variations in the length of the prepatent periods. The similar prepatent period in infections produced following the injection of the stages in black flies and those produced by the stages in tissues suggested a similarity in the asexual cycle in the infections produced by these two methods. If we allow for some asynchronicity of development and assume an asexual cycle of about five days or less we should expect to find small schizonts of the second and third generations at 5 and 11 days respectively as well as those of the first and second generation that were almost mature (Table IV, Figs. 8 to 11). A series of developing schizonts of known ages have not been seen but small schizonts were found five to six days after infection and slightly larger ones at seven to nine days after infection. Small schizonts as well as those that were almost mature were observed in tissues $10\frac{1}{2}$ days after infection. An asexual cycle of about five days would help to explain the occurrence of the peak parasitemia four to eight days after the parasites were detected in the peripheral blood. Gametocytes, as shown by blood transfusions, may live in peripheral blood for at least six days so that with an asexual cycle of approximately five days the peak parasitemia may include parasites of more than one generation. The host resistance might be expected to be more effective following one or two asexual cycles and tend to prevent the occurrence of even higher parasitemias following further generations. The relatively rapid increase in parasitemia in natural infections, the difficulty in producing artificial infections with parasites in tissues, the

rapid decline in the parasitemia, the absence of repeated peaks of parasitemia equivalent to the first, suggested that most of the merozoites developed into gametocytes rather than another generation of schizonts. As a schizont may contain more than a million merozoites relatively few of the former will be required to produce a noticeable infection, but as the schizonts occur in various tissues it is probable that few merozoites or schizonts are transferred when part of a tissue containing them is injected into another duck.

We agree with Huff and Wingstrand who thought that schizonts developed in macrophage cells. Our experiment showed that the blood of an infected duck contained, at certain times, a stage of the parasite that will produce the infection when injected into other ducks. It seems most probable therefore, that schizonts may develop in lymphocytic cells that may circulate for a time in the blood but lodge finally in some tissue where they continue to grow and change to macrophage cells as the parasites develop within them. A further indication that merozoites as well as sporozoites may be, for a time, either free or in some cell in the peripheral blood was given by the experiment in which blood, containing parasites, was injected into other ducks (Table V). In this experiment duck No. 718 presumably developed a new infection following the injection of blood. This blood most probably contained no developing schizonts that had arisen from sporozoites as the blood transfusion was made seven days after the donor duck had been infected at which time it probably contained parasites that had arisen from merozoites rather than sporozoites. It is of interest that Lastra and Coatney (12) have transmitted the related parasite, *Haemoproteus columbae* Celli and Sanfelice, by blood transfusions from infected to noninfected birds. Merozoites escaped presumably from schizonts in the tissues and grew into gametocytes in the blood. It might be expected therefore, that some merozoites that grow into schizonts might, for a time, be in the blood stream also. We have observed mature schizonts in the tissues only. It is possible we have overlooked their development in the blood but we think the evidence suggests that schizogony occurs more often in tissues and that they are in the blood only during their early development. The variety of tissues in which schizonts were found is a further indication that the merozoites from which they developed were at one time in the blood stream.

Ducks developed some resistance or tolerance when they were subjected to repeated infections but those with low grade parasitemias were not resistant to natural infections that were acquired six or more weeks after the first infection. The stimulation of the defensive mechanisms of the host by repeated infections appeared necessary to induce resistance. The importance of the spleen in these infections is indicated by the tremendous enlargement that occurs in it following infection, and if it is removed, the ducks harbored heavier infections for a longer period.

The splenomegaly, liver degeneration and hypertrophy, leucocytosis, and anemia are noticeable pathogenic effects of these parasites.

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APHID INFESTATIONS ON KATAHDIN AND ON A SEEDLING RESISTANT TO *MYZUS PERSICAE* (SULZ.), WITH TWO DATES OF PLANTING[1]

By R. H. E. Bradley[2] and R. Y. Ganong[3]

Abstract

In August under field conditions, on early planted plots, the number of *Myzus persicae* per plant was usually over five times as great on Katahdin as on B294-85; and the number of *Aphis abbreviata* was over 10 times as great on Katahdin as on B294-85. The differences in the aphid populations on the late planted plots of Katahdin and of B294-85 were similar, but not so great. There were no consistent differences in the numbers of *Macrosiphum solanifolii* on Katahdin and on B294-85. During August, the rates of increase of each species were similar for both varieties and dates of planting. The number of aphids per plant was many times greater on the early planted than on the late planted plots, the differences being greater between the Katahdin than between the B294-85 plantings. When the populations were expressed as aphids per unit leaf area, the distribution of each species on the top, middle, and bottom leaves was similar in all cases.

Introduction

During the past 10 years, the officers of the Field Crop Insect Laboratory at Fredericton and Woodstock, N.B., have been investigating the possibility of aphid resistance in potatoes. The population buildup of *Myzus persicae* (Sulz.) on a large number of potato varieties and potato seedlings has been followed under glass and under various types of cages in the field; in addition, the field infestation of thousands of potato seedlings has been investigated. To date, much of the work has concerned the finding of a method of testing the possible aphid resistance of large numbers of potato seedlings. In tests carried out under glass and under cages in the field, only one species of aphid, *M. persicae*, has been used; and the results have not always agreed with those obtained when plants are exposed to aphid infestation in the field. For example, Adams (1) repeatedly failed to infest *Solanum polyadenium* Greenm. under cotton cages with *M. persicae*; yet when *S. polyadenium* was grown in the field at Woodstock in 1946, large numbers of *M. persicae* were observed feeding on it.

Katahdin, a commercial variety that is widely grown in Eastern Canada, has consistently supported one of the largest aphid populations in these tests; and it has been used as a standard for comparing the aphid infestations of other varieties and of potato seedlings. A few varieties and some seedlings have usually supported lower populations than Katahdin, and are considered to be somewhat resistant to aphids (1). One of the most promising of these has been seedling Number B294-85 from the United States Department of Agriculture. To learn whether the resistance of B294-85 to *M. persicae* in

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these tests is indicative of a resistance to aphids in the field, an experiment was carried out at Woodstock, during the summer of 1950. Two dates of planting were used to determine whether field resistance to aphids was affected by the date of planting.

Methods

The experiment consisted of four treatments: early and late plantings of Katahdin, and early and late plantings of U. S. D. A. seedling Number B294-85. The dates of planting were May 24 and June 24. Treatments were replicated four times and arranged in a Latin square of 16 plots. Each plot contained 12 rows of potatoes and was 36 ft. square; plots were separated from one another by 18 ft. of fallow ground. The seed used was raised on the experimental propagation plot at Woodstock in 1949, and less than 1% of the tubers were infected with the leaf roll or the potato Y viruses. Planting was done by hand in machine-made drills; and the potatoes were cared for as if grown commercially, except that no insecticides were applied to control aphids. The plots were sprayed each week throughout July and August with a mixture of copper sulphate and calcium arsenate to control blight and biting insects. Sprays were applied with a six-row sprayer having three nozzles to a row and developing a pressure of 400 lb. per sq. in. at the nozzle.

Weekly aphid counts were made from the beginning of July to the end of the first week of September. The aphids were counted on a top, a middle, and a bottom leaf on each of 10 plants in each plot; the selections both of leaves and of plants were at random. The area of each leaf of the sample was estimated by means of a set of photographic leaf standards (4); in addition, the numbers of top, middle, and bottom leaves were counted on the 10 plants sampled from each plot. Thus, for each treatment the aphids on 120 leaves were counted, the areas of these leaves were estimated, and the number of leaves on 40 plants was recorded. With these data an estimate of the number of aphids per unit leaf area was calculated by means of the formula $\Sigma x/\Sigma a$, where a is the area of a leaf and x the number of aphids on that leaf. An estimate of the number of aphids per plant was obtained by means of the formula $\frac{1}{N}\left\{\bar{r}_1\Sigma x_1 + \bar{r}_2\Sigma x_2 + \bar{r}_3\Sigma x_3\right\}$ (Ref. (3)), where N is the number of plants from which a top, a middle, and a bottom leaf were selected; x_1, x_2, and x_3 are the numbers of aphids counted on single selected top, middle, and bottom leaves; and $\bar{r}_1$, $\bar{r}_2$, and $\bar{r}_3$ are the average numbers of top, middle, and bottom leaves per plant.

Results

The plants of both varieties appeared above ground three to four weeks after planting. No aphids were found during the first week of July, when over 100 leaves on each of the early planted plots were inspected. On July 10, however, aphids were found on some of the early planted plots and the average numbers per plant were: eight of *Macrosiphum solanifolii* (Ashm.) on Katahdin and on B294-85; five of *Myzus persicae* (Sulz.) on Katahdin; and

less than one on B294-85. Only one of *Aphis abbreviata* Patch was found on a Katahdin leaf. The following week, plants appeared above the ground in the late planted plots, and an aphid count on July 17 showed that all four series of plots were infested with *M. solanifolii*, *M. persicae*, and *A. abbreviata*. At this time the population was over 35 aphids per plant on the early planted plots and less than five per plant on the late planted plots.

Subsequent weekly aphid counts showed that the population continued to increase throughout July and until the last week of August. During July, *M. solanifolii* was the most abundant species, comprising over 75% of the aphid population. On July 31 there were over 200 aphids of all species per plant on each of the plots. During August both *M. persicae* and *A. abbreviata* became more numerous than *M. solanifolii*; and on Aug. 23 the total aphid population varied from over 33,000 per plant on the early planted Katahdin to about 900 per plant on the late planted B294-85. Throughout the summer only insignificant numbers of *Myzus convolvuli* (Kltb.) were found, and this species is not considered in the results.

Aphids per Plant

The weekly populations of *M. solanifolii* per plant are represented graphically in Fig. 1. *M. solanifolii* was the first species of aphid to infest the plots and apparently required only a few days for establishment. On July 10 there were about eight of *M. solanifolii* per plant on the early planted plots, yet no aphids had been found the previous week; similarly the first week that plants of the late planted plots appeared above ground, an aphid count showed there were about three of *M. solanifolii* per plant. The population of *M. solanifolii* was consistently greater on the early planted than on the late planted plots; however, the differences were small. Katahdin and B294-85 supported approximately the same numbers of *M. solanifolii* throughout the season.

The weekly populations of *M. persicae* and of *A. abbreviata* per plant are shown in Figs. 2 and 3 respectively. The results for these two species are similar in that the populations were much higher on Katahdin than on B294-85, and the populations were higher on the early planted than on the late planted plots. Fig. 2 shows that after aphid establishment the population of *M. persicae* on the early planted plots was usually over five times as great on Katahdin as on B294-85. Though the populations varied in the same direction on the late planted plots, the differences were only about half as great. The highest populations of *M. persicae* were recorded on the early planted plots on Aug. 23 and were 11,676 and 2547 per plant on Katahdin and B294-85 respectively. On the late planted plots the highest populations of *M. persicae* were recorded on Sept. 1 and were 583 and 276 per plant on Katahdin and B294-85 respectively.

Fig. 3 shows that after establishment the population of *A. abbreviata* on the early planted plots was consistently over 10 times as great on Katahdin as on B294-85; and though slightly smaller, the differences on the late planted plots were similar. The highest populations of *A. abbreviata* were recorded

on Aug. 23 and were 18,032 and 1471 per plant on early planted Katahdin and B294-85 respectively; and 250 and 90 per plant on late planted Katahdin and B294-85 respectively.

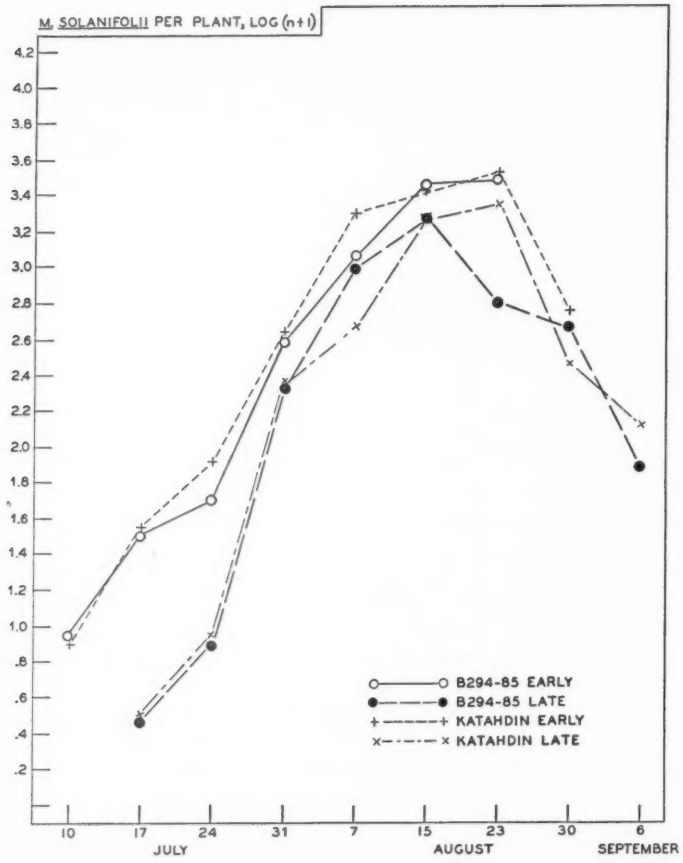

FIG. 1.

The differences in the populations of *M. persicae* and of *A. abbreviata* between the early and late planted plots were greater on Katahdin than on B294-85. During the first three weeks of August, the number of *M. persicae* was usually over 30 times greater on the early planted than on the late planted Katahdin, and only about 15 times greater on early planted than on the late planted B294-85. During August there were over 75 times as many of *A. abbreviata* on the early planted as on the late planted Katahdin; and about 25 times as many of *A. abbreviata* on the early planted as on the late planted B294-85.

In the graphs the numbers of aphids per plant have been transformed to their log $(n + 1)$ values to show differences between plots when the populations were low. Also, with this transformation the slopes of the curves indicate

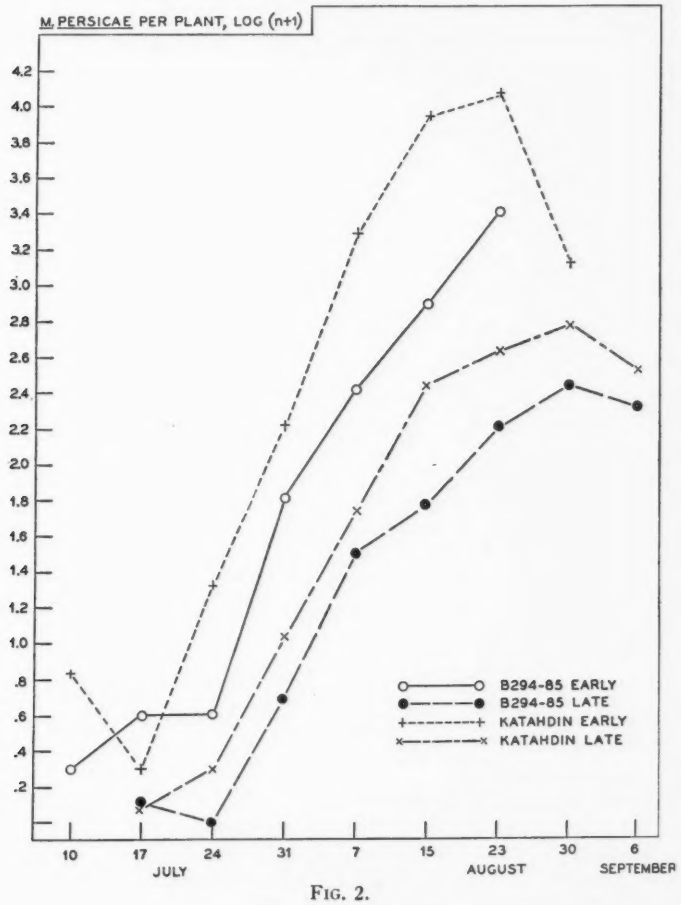

FIG. 2.

the rate of change of the population. In Fig. 2 the slopes of the curves show that the rate of increase of *M. persicae* between July 24 and Aug. 23 was approximately the same for both varieties and dates of planting; also, Fig. 3 shows that the rate of increase of *A. abbreviata* was approximately the same for both varieties and dates of planting between July 31 and Aug. 23.

The weekly records of the total aphid population per plant are shown in Fig. 4. Throughout August, the number of aphids per plant on the early planted Katahdin was over four times that on the early planted B294-85.

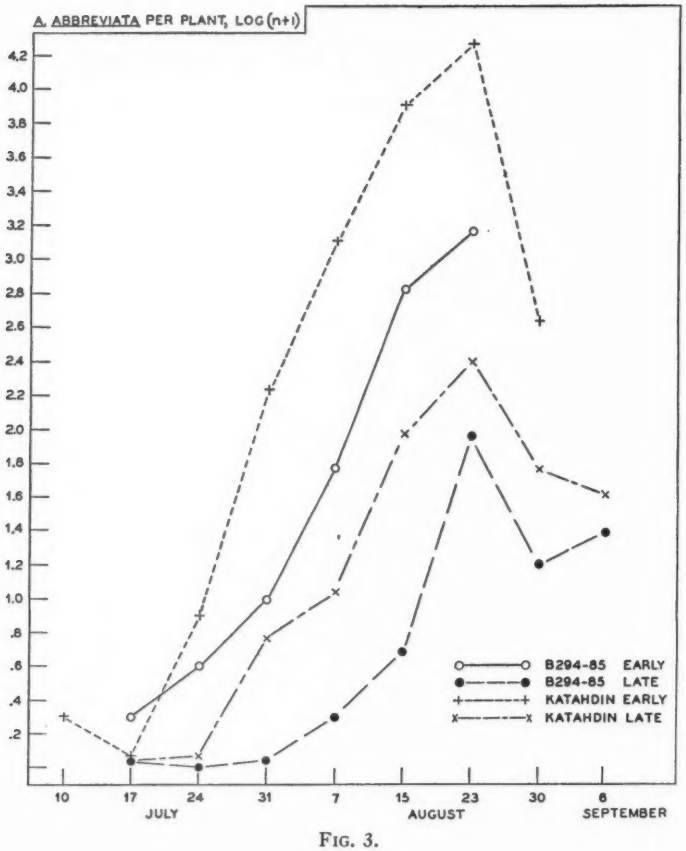

FIG. 3.

There were no consistent differences in the total population on the late planted plots. The slopes of the curves in Fig. 4 show that the rate of increase of the total population was approximately the same during August for both varieties and dates of planting. During the last week of August, all the plots suffered from an attack of late blight and symptoms of aphid injury developed; the early maturing B294-85 died within a week and further counts on it were not possible.

Aphids per Unit Leaf Area

When populations were expressed as aphids per unit leaf area, for both varieties and dates of planting, the distributions of species on the top, middle, and bottom leaves were found to be similar and were similar to those found on four varieties of potato at Woodstock the previous year (5). In general, the number of *M. solanifolii* per unit leaf area was greatest on the top leaves, the

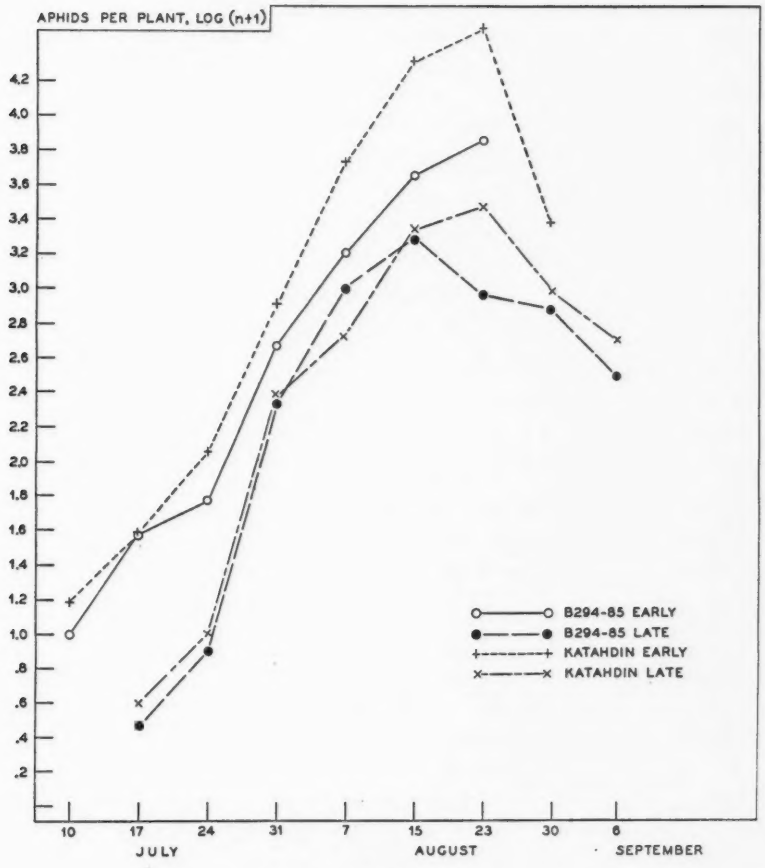

FIG. 4.

numbers of *M. persicae* and of *A. abbreviata* greatest on the bottom leaves. As the distributions of species on the top, middle, and bottom leaves were similar, only the results of one, early planted Katahdin, are represented graphically in Fig. 5.

Fig. 5 shows that on early planted Katahdin there were usually about twice as many of *M. solanifolii* per 10 sq. dm. of leaf area on the top leaves as on either the middle or the bottom leaves. Not only were the distributions of *M. solanifolii* similar, but the numbers per unit leaf area each week were similar for both varieties and dates of planting. The large differences between the populations of *M. persicae* and of *A. abbreviata* on Katahdin and B294-85, and between the early and late planted plots, were only slightly modified by expressing the populations as aphids per unit leaf area. Fig. 5 shows that

until the middle of August the populations of *M. persicae* and *A. abbreviata* per 10 sq. dm. of leaf area were over 10 times as great on the bottom as on the top leaves. During the latter part of August, the lower leaves suffered severe

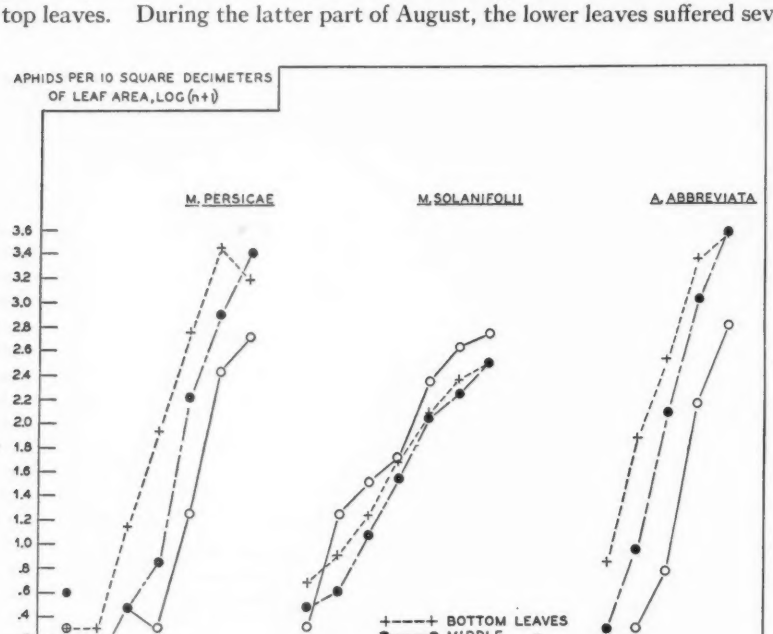

FIG. 5.

aphid injury and many died; during this time, aphids on the dying lower leaves moved to the middle and top leaves. The slopes of the curves in Fig. 5 show that the population of each species per unit leaf area increased at approximately the same rate on the top, middle, and bottom leaves.

Discussion

Mr. L. A. Dionne of the Fredericton laboratory demonstrated that, when plants under cages in the field were kept free from other insects and infested with equal numbers of *M. persicae*, lower populations developed on B294-85 than on Katahdin. The results of the experiment at Woodstock in 1950 showed that when the plants were exposed to infestation in the field lower populations of both *M. persicae* and *A. abbreviata* developed on B294-85 than on Katahdin; but no consistent differences were found in the numbers of

M. solanifolii on B294-85 and on Katahdin. Therefore a potato having resistance to infestation by one species of aphid need not be resistant to infestation by other species.

The factors that cause higher populations of both *M. persicae* and *A. abbreviata* to develop on Katahdin than on B294-85 remain undetermined. The sizes of leaves and numbers of leaves per plant were similar on Katahdin and on B294-85; and the plants of the two varieties appeared so similar that it was not easy to distinguish between them throughout July and August. The only noticeable difference between the development of Katahdin and that of B294-85 was that the seedling matured earlier than Katahdin. Because of the similarities in size, appearance, and rate of growth, the microclimatic conditions within plots of Katahdin and B294-85 must have been similar. The results of this field experiment show that the populations of *M. persicae* and *A. abbreviata* increased at approximately the same rate on Katahdin and on B294-85 during August. Though increasing at the same rate, the populations of *M. persicae* and *A. abbreviata* appeared to be about a week later in development on B294-85 than on Katahdin. This suggests that the large differences in the aphid populations on Katahdin and on B294-85 during August were caused by factors active at the time of aphid establishment in July. To determine these factors would require careful study during aphid establishment and unfortunately the aphid counts in this experiment were not detailed enough for this. Factors that may cause aphids to develop about a week later on one variety than on another and might cause large differences in the populations later in the season are: (*a*) a greater number of aphids infesting one variety than another because of differences in the catchment surfaces; (*b*) earlier initial infestation on one variety than on another; (*c*) a greater rate of mortality on one variety during establishment; and (*d*) a faster initial rate of reproduction on one variety than on the other.

Adams (1) suggested that resistance to aphids in potatoes would serve to control aphids as insect pests and should reduce the spread of aphid-borne viruses. At present the best means of controlling aphids on potatoes is the continual use of insecticides, and a degree of resistance to aphids in potatoes that would reduce the numbers of aphids until they no longer caused severe feeding injury to infested plants would be of great value to the grower. However, the assumption that a reduction in the numbers of potential vectors should reduce spread of aphid-borne viruses may be premature. Over 90% reduction in the aphid population on Katahdin throughout the season at Woodstock (2) with insecticides has not consistently reduced the spread of leaf roll. This suggests that aphid movement may be as important in the spread of viruses as the number of potential vectors. Though the population of aphids on Katahdin was over five times that on B294-85, aphid movement may have been greater on B294-85 and caused a greater spread of viruses. Thus, before the use of aphid resistance is advocated for potatoes, such resistance must be found and its effect on the spread of aphid-borne viruses ought to be investigated.

Acknowledgments

Mr. L. A. Dionne of the Fredericton laboratory demonstrated the aphid resistance of seedling B294-85, and we wish to acknowledge with thanks permission to refer to this work.

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POLARIZED LIGHT AND BODY TEMPERATURE LEVEL AS ORIENTATION FACTORS IN THE LIGHT REACTIONS OF SOME HYMENOPTEROUS AND LEPIDOPTEROUS LARVAE[1]

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Abstract

Larvae of the diprionid sawfly, *Neodiprion banksianae* Roh., the lasiocampid, *Malacosoma disstria* Hbn., and the tortricid, *Choristoneura fumiferana* (Clem.), were used to demonstrate the effects of heat and of plane-polarized light upon the photic orientation of immature insects. Photic orientation was shown to be primarily a result of internal temperature level. When larvae were heated sufficiently, they reversed the sign of their orientation. Larvae of the three species were sensitive to variations in the plane of polarization, and they used the polarization pattern of the sky to varying degrees in their orientation. *Neodiprion* larvae orientated primarily with reference to the polarization pattern when one was available. *Malacosoma* larvae and photonegative *Choristoneura* larvae appeared to orientate with reference to the position of the sun, but rotation of the axis of a "Polaroid" screen through 90° could change the direction of their travel by this amount. On the other hand, photonegative *Choristoneura* larvae subjected to a 90° shift of the axis continued to orientate with reference to the solar compass position when the sun was visible, even when their actions under the "Polaroid" showed that they could detect changes in polarization. The type of eye structure, the number of pairs of eyes, and the position of these on hypognathous and prognathous heads are considered to have some influence upon the different degrees of efficiency in orientation. Smoke and ice crystal cloud affected the orientation of the "Polaroid" axis that would produce a response, notably when the sun was obscured. Water droplet cloud had little effect, except in a complete overcast, under which polarization was disrupted.

Introduction

The classic experiments of K. von Frisch on the senses of bees, which have been summarized recently in book form (7), have aroused great interest among students of animal behavior. Of particular interest to those who study the light reactions of insects are the facts that bees make use of the polarization pattern of light from the sky (7), and that portions of their eyes may act as analyzers of polarized light (1). Search for these phenomena is being extended to other species of insects (10) and even outside the Class Insecta (11) but, so far as can be found in the literature, the work on insects seems to have been confined to the adult stage.

This paper reports the extension to immature Hymenoptera and Lepidoptera of observations of insect reactions to plane-polarized light. The results have been combined with a summation of earlier observations of the ordinary responses of these insects to light, and with observations of the effects of internal temperature level upon photic orientation in general.

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Materials

Larvae of forest insects were used as experimental animals. The diprionid sawfly, *Neodiprion banksianae* Roh., was selected as a representative of the Hymenoptera. The lasiocampid, *Malacosoma disstria* Hbn., and the tortricid, *Choristoneura fumiferana* (Clem.), were selected as representatives of the Lepidoptera. Where possible, larvae of the ultimate instar were used. Fifty larvae of each species were used, and the number of trials per larva varied from 5 to 10.

Larvae of all instars were used in previous observations of general light reactions which are summarized here as necessary background material. Some of the findings noted here have been described elsewhere (9, 12), or will appear in later publications. They were based on observations on 100 larvae of *N. banksianae*, 450 of *M. disstria*, and more than 1000 of *C. fumiferana*.

Observations were carried out both in the laboratory and under natural and artificial conditions outdoors. The various methods employed are described in the appropriate sections.

Results

Laboratory Light Reactions

This subsection is largely a summation of previous work. At room temperature, fully fed larvae of all feeding instars of the three species are positive both to diffuse light and to light from a point source. Prepupae are indifferent to light or, occasionally, weakly negative.

In photopositive larvae, there are differences both in the intensity of response and in the accuracy of orientation. Differences in the intensity of response are as follows: *Neodiprion* larvae are weakly positive, *Malacosoma* larvae are moderately so, and *Choristoneura* larvae are strongly photopositive. The distinctions apply both in a dark–light choice chamber fitted with diffuse, overhead illumination, and on a light board equipped with single or twin lights.

As noted, differences in accuracy of orientation are also observed. *Neodiprion* larvae orientate inaccurately to a point source. Their paths are often devious, and errors up to $\pm 30°$ may occur in their compensating turns. No true light compass reaction has been observed. *Malacosoma* larvae orientate accurately, moving directly towards a light source or performing a positive light compass reaction. However, when a second light is switched on during a compass movement, the larvae always strike a resultant course between the two lights (cf. 5). *Choristoneura* larvae orientate accurately, but a true light compass reaction is lacking in fully photopositive larvae.

Further differences appear when larvae are starved. Starvation increases the intensity of the response to both types of light in *Neodiprion* and in *Malacosoma* larvae, but it does not affect the sign of the reaction. On the other hand, although starved younger stages of *Choristoneura* remain positive, starved larvae of the last three instars show an increasing tendency towards

negative behavior with respect to point sources, and this becomes particularly marked in larvae of the last instar (12). Although these larvae become negative to point sources, they remain positive to diffuse light. In addition, their truly negative stage is preceded by a period during which they may respond to a point source either by moving directly towards it, or by performing a definite light compass reaction. Individual compassing larvae seldom strike a resultant course between two lights, in contrast to the behavior of *Malacosoma* larvae.

Heating larvae of all species changes their light reactions. When diffuse light is used, positive larvae placed in a dark-light choice chamber and heated, move into the dark, a reaction that is well known in insects (cf. 4). Reversal occurs over a range of a few degrees when groups of larvae are used, but only the mean temperatures need be listed here. The mean air temperatures at which larvae of the last feeding instar of each species reverse are: *N. banksianae*, 36.5° C., *M. disstria*, 35.5° C., and *C. fumiferana*, 37.9° C. The choice chamber used in this work always contained saturated air to guard against possible complications from excessive evaporation. Thermocouple measurements of the internal temperature of *M. disstria* larvae exposed in this chamber show a range 0.5–1.5 higher than the air temperature.

When larvae are provided with a point source of light and allowed to crawl on a heated brass plate, reversal to photonegative behavior occurs at the same temperatures as noted for the choice chamber. *Neodiprion* larvae exhibit a vague orientation. They do not move towards the light, but an individual may move in any direction between 90° and 180° from the light. *Malacosoma* larvae have two definite responses. First, they move at right angles to the light when they are heated to a range two or three degrees below their true reversal range. Second, they move directly away from the light when they are within their reversal range. The orientation errors are seldom as high as 10° in either movement. *Choristoneura* larvae show no tendency to move at right angles, but they move directly away from a light within their reversal range.

Field Light Reactions

Observations Without "Polaroid" Screens

Laboratory results may be used to interpret larval behavior observed outdoors. The key factor is an insect's internal temperature which, outdoors, depends partly upon the air temperature and rate of evaporation, and partly upon insolation, terrestrial radiation, and conduction (8, 13).

When *Neodiprion* larvae are below their reversal temperature, they move towards the half of the sky in which the sun is located. When above their reversal temperature, they move towards the opposite half of the sky. Under a completely clear sky, larvae may move in straight lines for short periods, but seldom maintain straight paths for more than 20-cm. distances. Shifts in track direction amounting to 30° occur, but are eventually compensated. When several individuals are exposed to uniform radiation conditions at the same time, their general direction of movement is the same, but their orientation errors and compensating turns do not occur in unison.

The same faulty orientation is observed when drifting smoke, or cirrus of either a fibrous or veil-like nature rises more than 30° above the horizon but does not obscure the sun. However, the compensating movements of a group of larvae frequently begin to occur in unison under this type of sky. It can be demonstrated that a larva need not be exposed to the others in order to establish synchronization with them. Vertical sheets of cardboard that isolate individuals but do not obscure the overhead sky do not interfere with the synchronization. The behavior breaks down, however, as suddenly as it appears, and often the movements are not noticeable for an hour or more at a time. The same synchronization may occur when smoke or ice crystal cloud obscures the sun but leaves some of the overhead sky clear.

When larvae are artificially shielded from direct sunlight but are still exposed to the sky, they are generally cool enough to be photopositive. It would be expected that they move towards the direction in which the sky appears brightest to an observer, but this does not always occur. Whatever the direction of travel, continuing changes in the paths may still be observed, and, if smoke or ice crystal cloud appears, synchronous turning similar to that noted above begins.

Malacosoma larvae show three basic directions of travel under natural conditions when they are on a smooth surface. If the sun is not obscured, larvae move towards its compass position when their internal temperature is below 32–33° C. They move at right angles to the direction of the sun when their temperature is in this range (mean internal temperature, 32.7° C.), and directly away from the solar position when their temperature rises to 35.4° C. or more (9). The track of any individual is very straight, and its error in orientation with respect to the position of the sun is seldom as much as 10°.

When the sun is obscured by smoke or cloud so that its disk is not visible to an observer, groups of larvae may still be affected by the substrate temperature, but their directions of travel with respect to the position of the sun are not so rigid. Nevertheless, the direction of travel of an individual is rigidly maintained if only the sun is obscured. On the other hand, if the smoke or ice crystal clouds move into the overhead sky while the sun is obscured, sudden slight directional changes begin to recur in the paths of travelling larvae. Clouds composed of water droplets do not seem to be associated with these recurring changes in direction to any marked degree.

If *Malacosoma* larvae are shielded artificially from direct sunlight but left exposed to the sky, their behavior, for the most part, is similar to that described above. They maintain steady directions of travel under clear sky or under broken, water droplet clouds. Smoke or ice crystals are associated with the frequent changes in direction noted above. A sky completely veiled by smoke or ice crystals, or a solid overcast of water droplet cloud, is associated with movement towards the direction of the brightest sky, and the frequency of directional changes is reduced to one every 2 or 3 meters.

Positive and negative *Choristoneura* larvae behave like those of *Malacosoma*, whether or not the sun is visible.

Observations With "Polaroid" Screens

Polarization of sky light is a phenomenon due to scattering of radiation. The plane of polarization is fixed by the positions of the sun, the observer, and the point observed. Ordinarily, the percentage of light that is polarized increases from a minimum at or near the solar and antisolar points to a maximum midway between them, and the midway maximum, in turn, increases with the altitude of the midpoint observed. This is the normal polarization pattern of the sky. Phenomena that increase the amount of reflected light generally decrease the percentage of polarized light.

All observations were made outdoors. A disk of "Polaroid" 23 cm. in diameter was used. The straight line joining the solar and antisolar points was used as a base line to which the various compass positions of the axis of the "Polaroid" could be referred directly and expressed as angles when the disk was held flat. Thus, when the "Polaroid" axis was parallel to the base line its compass direction was noted as a "Polaroid" axis angle of zero degrees (P 0°). When the disk axis was at right angles to the base line, its position was recorded as P 90°, and any other angle between zero and 90° was noted in similar fashion. The setting of P 90° was that the one that usually had the least effect upon the appearance of the natural polarization pattern of the sky when this was viewed with and without the "Polaroid", but occasionally other axis settings had to be used. The setting of P 0° usually had the most effect upon this pattern when the sky was viewed through the disk. The naturally darker areas were lightened, and the naturally brighter areas usually were darkened considerably.

At the beginning of an observation, individual larvae were allowed to move freely on a smooth substrate that had a uniform surface temperature. Within a few seconds, they established a direction of travel that depended upon their temperature. The observer then examined the overhead sky with the "Polaroid" to determine the orientation of its axis that had the least effect upon the existing polarization pattern. When the axis of the disk was correctly orientated, the "Polaroid" was centered over a moving larva and held about 2 cm. above it. If this were done gently, and the sky conditions remained unchanged, the presence of the disk had no effect upon the insect's direction of travel. Ten seconds were usually sufficient to determine the presence or absence of response. When there was no response, the disk axis was shifted rapidly to another compass position and held steady there to observe the effect.

Observations were made during the second week in June, 1951, at Sault Ste. Marie, Ont. They were confined to the periods 0800–1100 hr. Eastern Standard Time and 1300–1600 hr. E.S.T. Between 1100 and 1300, the solar elevation was too great to permit observations of the effects of the solar position upon the orientation of some of the larvae. Prior to 0800, remnants of nocturnal inversion smoke or cloud were too frequent, and, after 1600, there was a danger of too much convective or turbulent cloud.

No response was obtained at the axis setting of P 90° when the sky was clear and the sun was in view. *Neodiprion*, *Malacosoma*, and photopositive *Choristoneura* larvae rarely responded to axis shifts of less than 90° from this basic position. A 90° shift in the direction of travel occurred when the axis was moved from P 90° to P 0° and held in the new position. The new direction of travel was maintained as long as the disk was held at the new setting, and the original orientation was resumed when the axis was returned to its first setting, or when the "Polaroid" was removed.

All species responded in either of two ways to the setting at P 0°. The first type of response was immediate and dramatic. The larva under observation stopped, lifted its head and searched, then returned the forepart of its body to the ground at an angle about 45° from its original heading. It then resumed motion, making the adjustment in direction through the final 45° as it moved. Adjustment was complete within about five body lengths. Return to the original track was sometimes made in as marked a fashion when the disk was moved again, but frequently it was an adjustment of the type described below.

The second type of response was not immediate. Sometimes nothing happened for as long as 10 sec. after the shifting of the axis. Eventually, the larva gradually began to turn from its course and moved in a smooth curve into the new direction of travel. Occasionally, there was some hesitation near the beginning of the change in direction, but the insect never actually stopped. Once begun, this type of reorientation required up to 20 sec. to complete.

Clockwise or anticlockwise rotation of the "Polaroid" axis produced similar shifts in direction in 80% of the tests. The remainder of the larval responses took place in the direction opposite to the rotation of the disk. An individual might respond either way.

No noticeable discrepancies in the results occurred if the sun were in view when stratocumulus or cumulus clouds composed of water droplets were in the sky. The first discrepancy in response was observed between *Neodiprion* on the one hand and *Malacosoma* and positive *Choristoneura* larvae on the other, when a fibrous cirrus patch covered the sun. The patch was not dense enough to obscure the sun, but it glowed brightly throughout its area of 12 × 12 solar diameters. *Neodiprion* larvae no longer responded to P 0° in any consistent fashion. This setting still produced most of the responses, but they were sometimes turns of 45°, instead of 90°, and sometimes no change in direction could be obtained. When there was no response at P 0°, one could be obtained at P 90°.

Several *Neodiprion* larvae had been moving together on a sheet of brown cardboard, and it was noted that the synchronous turning described in the preceding section began when the cirrus crossed the sun. The turns were of small angle, ranging from 10 to 30°, but the interesting point was that a larva under the "Polaroid" and the remaining, fully exposed larvae all turned at once and by approximately the same amount. The exposed larvae were all heading into the same quadrant, and, each time they turned, all

turned in the same direction. The larva under the "Polaroid" (P 0° setting) was headed in a direction differing by 90° from the general trend of the others. Therefore, its turns, although synchronized, were made to different compass points. Nevertheless, in terms of bearings, right or left, they were the same as those of the other larvae. Each larva on the board was tested in this way, and the results could be duplicated, although sometimes the P 90° setting had to be used, until more cirrus appeared in the overhead sky. At that time, it was difficult to keep the proper axis setting for more than a few seconds, since it flicked rapidly between P 0° and P 90°, and occasionally appeared at intermediate angles.

While *Neodiprion* behaved in this fashion, the other species continued to orientate as usual, and responded to the P 0° setting. Similarly, when smoke drifted at 30–50° above the horizon but did not cross the sun, larvae of the other species reacted as usual, but *Neodiprion* larvae began synchronous turning, and their responses under the "Polaroid" were as described above.

The next discrepancy in the general response to P 0° occurred when dense smoke obscured the sun but not the overhead sky. When this happened, the P 90° setting produced responses most frequently. Furthermore, directional shifts of only 45° began to appear in the *Malacosoma* and *Choristoneura* individuals under "Polaroid", as well as in *Neodiprion* larvae. When the drifting smoke began to cover the sky as well as the sun, P 90° remained the most successful setting for a time, but, finally, no response could be obtained at any setting. By this time, the smoke was uniformly distributed in the overhead sky.

During the time that the smoke was covering the sky, larvae of all species began to change their directions slightly but frequently, whether or not they were under the disk. The behavior of the lepidopterous species was similar in some respects to that of *Neodiprion*, in that individual larvae frequently changed at the same instant, but there was no apparent order to the direction of the turns. It is of interest that the only shift of more than 90° that was ever observed occurred under a partially smoky sky when a *Malacosoma* larva under "Polaroid" turned through 180°.

Dense cirrus or other types of clouds composed largely of ice crystals produced the same effects as the smoke when they obscured the sun. Water droplet clouds did not, as long as they contained three or four breaks, each of which averaged four solar diameters. Rarely, an apparently solid overcast formed by stratus, with or without stratocumulus, had no effect upon the usual response to P 0°. If such a cloud were viewed through the "Polaroid" it was found to contain numerous gaps about one-third of a solar diameter in width and about five solar diameters long. A really solid water droplet overcast, which also obscured the sun, completely disrupted the response to "Polaroid" rotation.

Description of photonegative *Choristoneura* larvae has been reserved until this point because of a marked difference in response to "Polaroid" rotation. When the sun was in view, photonegative larvae moved away from its compass

position. Rotation of the "Polaroid" to P 0° produced a momentary reaction, but it was not prolonged. A larva usually paused and searched, and sometimes one would then strike off at an angle differing by 30–45° from its original track for a distance of some three body lengths, but this action was followed by a second pause. After this, the original direction was resumed and maintained. Actually, a slight shift of 10 to 20° from the original P 90° setting produced a more marked response than a shift from P 90° to P 0°. With the smaller shifts, the larva would curve smoothly into a new direction for 8 or 10 body lengths, then suddenly slacken its pace, halt and search, and return to the original orientation. When the sun was fully obscured, these larvae reacted much more strongly to "Polaroid" rotation and behaved similarly to larvae of the other species.

The preceding observations were made when the solar position was visible to the observer, whether or not the sun was obscured. Therefore, presumably the position was available to larvae of all species if they could detect it. To test their responses when the solar position was not available, they were placed so that the sun was hidden by the laboratory building. These observations had to be made during the earlier periods of the mornings. The observer could still determine the compass direction of the sun by moving to the edge of the building, so the same base line was used for reference.

The setting at P 0° produced 90° shifts in the tracks of all species under a clear sky, just as in the presence of the sun. Clouds composed of water droplets did not affect this reaction. Cumulus clouds composed largely of ice crystals appeared on the antisolar side of the observer during one series of tests, and when their tops reached to within 50–60° above the horizon, the P 90° setting began to produce responses in *Malacosoma* and *Choristoneura* larvae. Placed in the open sunlight, these larvae began to respond to P 0°. Similarly, dense patches of cirrus or smoke overhead were associated with responses to the P 90° setting when the sun was behind the building.

Discussion

The preceding observations have demonstrated that the late instar larvae of the three species are sensitive to the plane of polarization of light from the sky. Therefore, the results extend the known range of this phenomenon from adult Hymenoptera (7, 10) possessing compound eyes to larval forms of two orders possessing two types of simple eyes.

Although responses to "Polaroid" rotation under ideal conditions are very marked, it has been shown that some sky conditions that affect the normal polarization pattern of the sky light can influence results, sometimes in unpredictable ways. Cloud types are seldom noted in ordinary biological observations, and it is hoped that the distinctions made here between water droplet clouds and ice crystal clouds or smoke will be of value to any who wish to observe responses of other insects to polarized light. Attention should also be paid to haze, which was not present during this series of observations, for

it may affect the polarization pattern or amount. Other sky conditions not available during our work may produce still other discrepancies.

In the present work, there was not enough "Polaroid" available to construct an "artificial eye" of the type described by von Frisch, Therefore, when the polarization pattern was changing rapidly, it was not always possible to determine effective positions of the "Polaroid" axis. Furthermore, when these were found at unusual angles, it was not often possible to understand how they influenced the observed larval shifts in direction.

Prepupational travel has always interested entomologists, but the manner in which direction of travel is established has raised recurring questions. Not all of these have yielded to explanation in terms of light compass reactions. Many larvae which leave their host plants in large numbers within a short space of time engage in prepupational travel, but sometimes they appear to act more like a milling throng than an orderly group when they are observed under natural conditions.

The present work has clarified one aspect of the establishment of direction of travel by pointing out the dependence of the general orientation upon the internal temperature level. The tendency of many species to reverse the sign of their orientation to light when heated or cooled in the laboratory is well known, but it also may be used to interpret field behavior. An assumption that the throngs of larvae leaving their hosts are using some aspect of light as a guide, and that their reaction to it depends upon how warm or cool they are, should assist in the interpretation of their movements. The substrate upon which such larvae move in the field is often a patchwork of substances of varying heat capacities. Consequently, under apparently uniform insolation, different intensities of reradiation and degrees of conduction to the bodies of different larvae will provide each with a varying amount of heat. This will affect the basic orientation. When allowance is made for further complication by shadow patterns, a certain order may be extracted from apparent chaos in the field. It is becoming clear that there are very few truly random movements in insect travel.

At first thought, the observed sensitivity to plane-polarized light suggests a need for reconsideration of the terms of reference for light compass reactions, since, in both laboratory and field observations, compass movements are performed with apparent reference to point sources of light. Nevertheless, existing theory (5) still seems adequate, since it classifies the reactions of animals to intensity and/or direction of light in a graded scale. The three species that have been described here provide good examples of such a graded series, and further consideration of their different levels of response appears below.

The three species are characterized by one obvious division in their types of visual equipment. The *Neodiprion* larva has a single pair of eyes, set about halfway down the sides of the epicranial plates. Each eye bears a general resemblance to the dorsal ocellus of an adult insect. The lepidopterous larvae have six individual eyes on each side of the head. The positions vary between

the two species, but each eye has the general structure of an ommatidium of a compound eye, although it is covered by a corneal lens that may vary in the complexity of its partitioning, so that there appear to be three sublenses over some of the eyes.

Outdoors, in the absence of direct sunlight, all species respond so quickly to rapid changes in the polarization pattern of the sky that they appear to be disorientated at times, until their behavior is analyzed with the aid of a sheet of "Polaroid". The results of this analysis suggest that they may be using the same basic mechanism to respond to changes in the plane (and perhaps in the amount) of polarization. In this connection, polarization analyzers have been demonstrated both in the eye of the bee (1) and in the eye of the xiphosuran, *Limulus* (11). It is particularly interesting that maximal and minimal response rates in the *Limulus* eye occurred with polarization planes 90° apart, since this is the angle that most frequently produced a definite response in the three species dealt with here.

The first division in the types of responses comes between *Neodiprion* and the two lepidopterous species. Although all species respond to sudden changes in the natural polarization pattern in the absence of direct sunlight, larvae of *Neodiprion* never rise above this level, even when the sun is present. The other two species are able to maintain steady directions of travel under a clouded sky when the sun is not completely obscured, and under a clear or water-droplet-clouded sky if the sun is obscured.

Neodiprion larvae are extremely sensitive to the plane of polarization. They travel straighter paths under the sky than they do in laboratory light tests. Few would claim that these larvae perform light compass reactions, yet, within their limited ability, they clearly direct their travel with reference to the apparent brightness pattern of the sky which is revealed by a polarization pattern not always visible to the unaided eye of an observer. Their simple, adultlike ocelli appear to be the obvious weakness in their orientation apparatus. Nevertheless, it is possible that their inability to orientate precisely is as much a function of the number and location of these ocelli as it is of their type.

It is evident that the presence of the sun exerts a steadying influence upon the directions adopted by the lepidopterous larvae. However, even when the sun is present, *Malacosoma* and positive *Choristoneura* larvae can still be shifted 90° from their original directions by a shift of the "Polaroid" screen. Clearly, the larvae make use of the area of the sky in which the sun is located when it is available to them, but the fact that they are so influenced by "Polaroid" suggests that this ability to "use the sun" is simply a more highly developed ability to detect areas differing in apparent brightness and to use such patterns for orientation. The greater facility that the lepidopterous larvae possess in this respect presumably stems from the increased number of their stemmata, and possibly from the ommatidiumlike structure of these.

The next division in types of responses comes between photonegative *Choristoneura* larvae and the other photic types of the lepidopterous species.

When the sun is visible, photonegative *Choristoneura* larvae simply do not shift if the "Polaroid" axis is turned. There is no doubt that they detect changes in the axis, for their momentary responses have been described. Nevertheless, they maintain their orientation with respect to the compass position of the sun, in contrast to the behavior of the others.

This type of reaction seems to require a primitive type of form vision for its inception; a visual acuity certainly less than that exhibited by compound eyes, but at least well enough developed to perceive point sources as spots or disks of light. This has already been suggested (12) and, although it is more in accord with the mechanism underlying light compass reactions found in adult insects, it is not in line with the level of visual acuity assigned to caterpillars. *Malacosoma* larvae appear to be more typical caterpillars. Their reactions seem to be based largely upon comparisons of intensities, as is suggested also by their reactions to additional light sources during compass movements in the laboratory.

For a time it was considered that differences in the number of subdivisions of the corneal lenses of *Choristoneura* stemmata might be involved in any explanation of its anomalous behavior (12). Larvae of the first three instars do not show anomalous reactions and, in these, only the sixth pair of eyes have tripartite lenses. Larvae of the later instars have corneal lenses which are all tripartite, but some of them are the fused, unitary type described by Dethier (2).

Dethier (2, 3) examined the dioptric apparatus of the stemmata of *Isia isabella* A. & S., an arctiid, and demonstrated that tripartite corneal lenses, with their underlying divided crystalline lenses, were quite inefficient in image formation, partly because the three axes present in each system were seldom aligned. However, the shape of the head and the placement of the eyes upon it may prove to be a factor.

Many lepidopterous larvae, including those of *Malacosoma*, have a hypognathous head. On the other hand, the head of the tortricid, *Choristoneura*, is prognathous. The epicranial area is flat, with sharply curved outer edges, and the eyes are set along these curves, so far forward that the second pair of eyes faces almost directly ahead. Tripartite lens systems of eyes set on a sharply curved surface may possibly function as prism systems, so that overhead vision might be better than in most lepidopterous larvae. In any event, the forward vision should be considerably better than in most lepidopterous larvae.

These are only tentative suggestions based on observations of the structure of the heads of the species. They are worth keeping in mind, but it also should be recalled that the positive larvae of *Choristoneura* have the same external structure as do negative larvae, yet they act like *Malacosoma* larvae. In the face of this fact, further speculation concerning external structures is not very productive. The visual apparatus and its placement on the prognathous head of *Choristoneura* certainly are concerned with varying levels of response, but there appears to be a more basic reversible internal mechanism. Although this is not necessarily located in the eyes, it clearly produces its results through them.

The whole question of the effect of temperature upon the photic orientation of insects needs to be examined from a physiological standpoint. What takes place inside an insect that enables it to respond differently to the same photic stimulus at different temperature levels? (In this connection, we have found that heating only the heads of the three species would not produce reversal. The bodies had to be heated. Heating the bodies alone produced reversal.)

We have shown three levels of response in the orientation of the three species to light. The separation of the three levels has provided further evidence of differences between organisms performing a light compass reaction by comparisons of intensity and organisms that perform this movement by virtue of this ability plus the use of a higher level of visual acuity. The larva of the spruce budworm, *Choristoneura fumiferana* (Clem.), has been shown to be capable of orientations which suggest that it uses its visual apparatus more efficiently than most caterpillars do. J. Franz (6) has presented results that show that the European tortricid, *Cacoecia murinana* (Hb.), is very similar to the spruce budworm in several respects, and it would be interesting to examine the light reactions of this species.

Conclusions

1. The basic photic orientation of the larvae tested is a function of their internal temperature. When they are cool, they are photopositive, and when they are too hot, they are photonegative. This can be demonstrated both in the field and in the laboratory, using either point sources or diffuse light. Apparent orientation with respect to the compass position of the sun is dependent upon internal temperature, but there are varying degrees of efficiency in this orientation.

2. Use of a "Polaroid" screen shows that the test species in any photic state are sensitive to the plane of polarization of light, and that they use the polarization pattern of the sky to varying degrees in establishing their orientation. Turning the "Polaroid" axis through 90° produces the most marked changes in direction of travel.

3. Smoke or ice crystal cloud in the sky affects the orientation of the "Polaroid" axis that produces the response. *Neodiprion* larvae respond to a different axis direction under these conditions whether or not the sun is obscured, whereas *Malacosoma* and *Choristoneura* larvae respond to a different axis direction only if the sun is obscured.

4. *Neodiprion* larvae respond primarily to the polarization pattern of the sky when it is available, presumably through its production of a pattern varying in apparent brightness. When the amount of polarization is small, they respond to intensity gradients. The other species possess this basic level of response, but they are also able to orientate with reference to the position of the sun.

5. Further differences appear in this ability to orientate by the solar position. *Malacosoma* and photopositive *Choristoneura* larvae still respond to shifts in the "Polaroid" axis when the sun is present, so that they apparently

are influenced to some degree by broad patterns of varying brightness. Negative *Choristoneura* larvae are not influenced by "Polaroid" when the sun is visible. Although they give evidence of being able to detect polarization shifts, they continue to orientate with reference to the solar compass position.

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STUDIES ON THE ENDOPARASITIC FAUNA OF TRINIDAD MAMMALS

VIII. PARASITES OF MARSUPIALS[1]

By R. W. Wolfgang[2]

Abstract

Two species of Trematoda and 17 of Nematoda were recovered from three species of opossums. Of these, a trematode (*Zonorchis philanderi*), and seven nematodes (*Cruzia cameroni, Subulura trinitatis, Camerostrongylus didelphis, Philostrongylus philanderi, Spirocerca cylicola, Trichuris urichi,* and *T. reesali*) are new.

Of the four species of marsupials present in Trinidad, no specimens of *Marmosa chapmani* (Allen) or of *M. carri* (Allen and Chapman), and only a single individual of woolly opossum or *Philander trinitatis* were examined. However, 15 specimens of *Didelphis marsupialis* were available; 14 of these belonged to the subspecies *D. m. insularis* (Allen) and one to the subspecies *D. m. karkinophaga* (Zimmerman). This latter species is the crab-eating opossum and is comparatively rare, being predominantly a mainland form. *Didelphis marsupialis insularis* is known locally as the manicou and is present on some of the other West Indian Islands. These specimens were collected between 1932 and 1949 by the late Professor Urich, Prof. T. W. M. Cameron, and Mr. M. R. Reesal either directly from the opossums or from formalized entrails. The material consisted of two species of trematodes and 17 of nematodes all being preserved in formalin. No cestodes or thornyheaded worms were present.

Trematoda

Rhopalias coronatus (Diesing, 1850) Stiles and Hassall, 1898

Fourteen small tightly curled distomes were found in the small intestine of *Didelphis marsupialis karkinophaga*. Fig. 1 is a drawing of an in toto mount from ventral view. The trematodes measure from 3.79 to 4.24 mm. long by 0.89 to 1.18 mm. wide. The ratio of the anterior to the posterior end varies from 1 : 2.6–4.5. The shape of the anterior end is quite variable and may appear inflated as in the figure or simply oval as in the drawings of Caballero *et al.* (6) and Braun (4). The unique proboscides lie on either side of the pharynx and oesophagus (Fig. 2), measuring 0.56 to 0.77 mm. long by 0.18 to 0.21 mm. wide. Spines, which measure 0.042 to 0.052 by 0.02 mm. for the larger specimens, were never found in numbers greater than nine per worm; the larger spines (Fig. 2) are at the posterior end of the proboscis. The oral sucker is small, sub-ventral, and triangular, measuring 0.21 to 0.24 mm. wide by 0.168 mm. long. Immediately anterior to it (Fig. 3) is a raised

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portion bearing two rows of six spines each, and another set of six spines is located lateral to these on either side or presenting a formula of 6–12–6. This seems to be constant for the species.

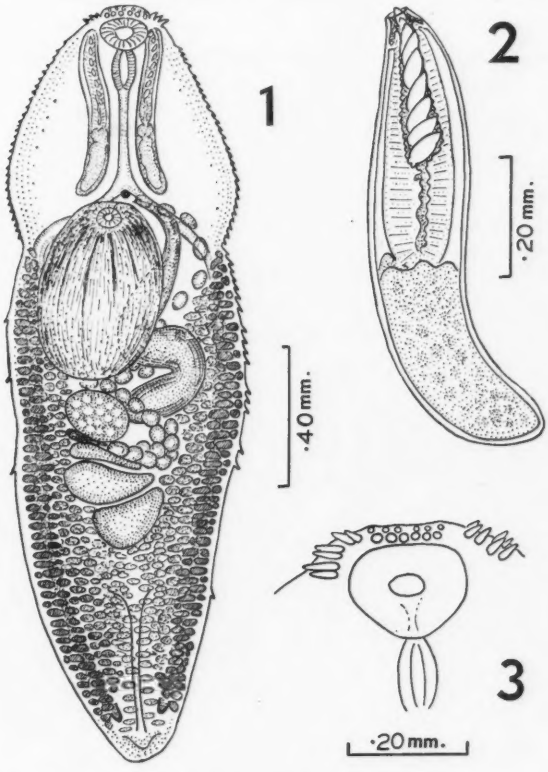


FIG. 1. Ventral view of *Rhopalias coronatus*.
FIG. 2. Proboscis of *R. coronatus*.
FIG. 3. Arrangement of preoral sucker spines of *R. coronatus*.

The acetabulum is a strong muscular organ situated slightly to the left side of the worm. In cross section it is flask-shaped and sets at an acute angle anterior to the ventral surface. The ratio for the oral sucker and acetabulum averages 1 : 3. The cirrus is large and terminates at the same point as does the uterus, after passing to the right of the acetabulum at the simple genital pore. The genital organs are similar to those described previously for the species. Eggs are few in number and measure 90 to 100 μ by 60 to 80 μ.

Rhopalias coronatus has previously been described from this host from Brazil (4, 5). My specimens differ from previous descriptions of *R. coronatus* as follows: shorter length; anterior to posterior body ratios less than described

previously; fewer proboscis spines; proboscis shorter but reaching the acetabulum; cirrus pouch smaller. Since these differences are regarded as insignificant, the specimens are assigned to this species.

This genus of trematodes is restricted to American marsupials and the four species recorded may be identified as follows:—

1. Proboscides long, spines large, arranged in a row (9 to 12 spines) *R. coronatus*
   Proboscides short, spines large or small but not arranged in a row.....2
2. Spines small and numerous.....*R. horridus*
   Spines larger (73 to 125 μ) 7–10 spines.....3
3. Eggs numerous (spines 73 μ in group of 7 to 8).....*R. baculifer*
   Eggs few (spines 125 μ, group of 10 near the tip.....*R. macracanthus*

Zonorchis philanderi sp. nov. (Figs. 4 and 5)

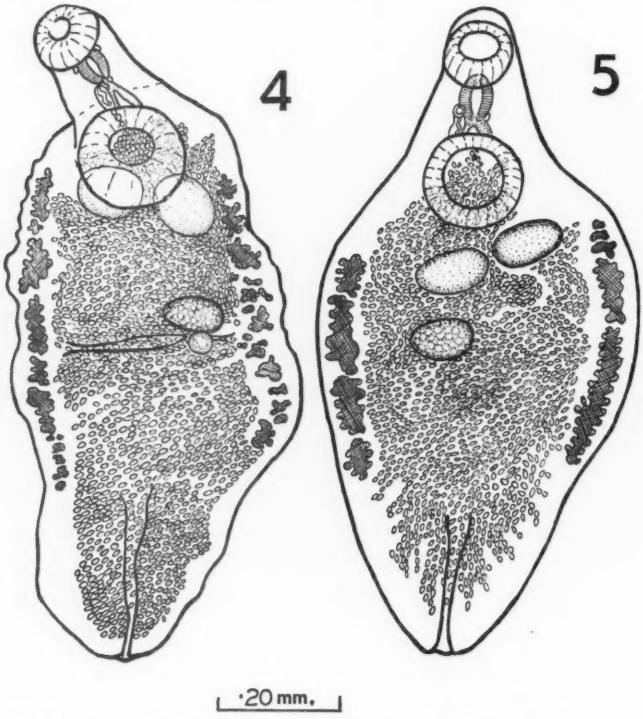


FIG. 4. Ventral view of *Zonorchis philanderi* ovary on the right.
FIG. 5. Ventral view of *Z. philanderi* ovary on the left.

Four specimens recovered from the bile ducts of *Philander trinitatis* were in mountable condition. The worm is leaf-shaped, small, and filled with eggs. It is 2.01 to 2.21 mm. long by 1.22 to 1.32 mm. wide. Relaxed specimens

have a neck anterior to the acetabulum. The oral sucker is round, muscular, and measures 0.24 mm. in length. The pharynx is 0.14 by 0.12 mm. and the oesophagus is very short. The caeca branch close behind the pharynx and disappear in the mass of eggs and vitellaria. The acetabulum is 0.24 to 0.39 mm. from the anterior end and measures 0.33 to 0.36 mm. by 0.28 mm. It may be round or oval transversely. The ratio of oral sucker to acetabulum is 1 : 1.4–1.5. The testes are smooth and oval in shape, subequal, and situated side by side in the region behind the acetabulum. In one specimen (Fig. 4) they are situated beneath the acetabulum and are always separated by the ascending branch of the uterus. The cirrus pouch measures 0.1 by 0.06 mm. and lies to the right side of the pharynx at the genital pore. The ovary is smooth, oval, and situated behind one of the testes. In two specimens it was found to be on the right (Fig. 4), in the other on the left (Fig. 5). It lies about midway between the anterior and posterior ends of the worm. The seminal vesicle and mehlis gland are situated behind the ovary and the yolk ducts connect in this area. The vitellaria are lateral, extending from the level of the acetabulum or anterior to the testes, to the posterior third of the worm. The arrangement of small follicles in linear groups may indicate that they lie lateral to the caeca. The uterus has ascending and descending lobes filling the entire area behind the genitalia, and after passing between the testes, is thrown into several folds beneath the acetabulum. The oval eggs measure 26 μ to 33 μ by 18 μ to 19 μ.

Nine species of *Zonorchis* have been described (10), of which two are from marsupials: *Zonorchis allentoshi* Foster, 1939 (10), from *Philander laniger pallidus* in Panama, and *Z. goliath* Travassos, 1945 (21), from *Didelphis aurita* in Brazil.

The differences between these and the present forms are shown in Table I.

TABLE I

Species	Length, mm.	Ratio, sucker–acetabulum	Uterus position
Z. allentoshi	3.0 – 5.7	1 : 1.9 – 2.0	Between testes direct to genital pore
Z. goliath	9.13 – 9.14	1 : 1.68 – 1.72	Between testes direct to genital pore
Z. philanderi sp. nov.	2.01 – 2.21	1 : 1.50 – 1.58	Between testes folding under acetabulum

The Trinidad species is the shortest, has the lowest sucker–acetabulum ratio, and has folds of the uterus under the acetabulum and testes behind or below the acetabulum. It is accordingly regarded as a new species with the name of *Zonorchis philanderi*.

Nematoda

Aspidodera raillieti Travassos, 1913

A large number of specimens was always found in the large intestine of *Didelphis*. Males and females are about the same size—5.62 to 7.85 mm. in length for males, and 5.13 to 8.95 mm. in length for females. Both sexes have a maximum width of 0.45 mm. This species seems to be identical with *Aspidodera raillieti* Travassos, which has previously been recorded from the mainland of South America in *Didelphis aurita* Wied. and *Metachirops opossum* (Temm) (17) as well as from *Philander laniger pallidus* (Thomas) in Panama (10).

Cruzia cameroni sp. nov.

This worm is the most common species in the present collection, from both species of *Didelphis*. The male measures 12.7 to 13.7 mm. long by 0.48 to 0.54 mm. wide. The female is 9.15 to 18.25 mm. long, averaging about 15.0 mm. by 0.41 to 0.65 mm. wide. The oesophagus measures 2.4 to 2.7 mm. in the male and 2.1 to 2.3 mm. in the female. It is divided into four parts (Fig. 7): a short pharynx with three rows of 13 to 17 teeth corresponding to the triradiate character of the oesophagus, a long corpus, a short prebulbular swelling, and an oesophageal bulb. The nerve ring surrounds the oesophagus at 0.54 to 0.63 mm. from the anterior end, and the excretory pore is located 1.08 to 1.26 mm. from the anterior end (Fig. 7).

There is an anterior intestinal diverticulum, 0.06 to 0.99 mm. long, which never reaches more than half way to the nerve ring.

The female tail (Fig. 6) tapers gently and measures 0.86 to 1.29 mm. The nonsalient vulva is situated about the middle of the worm, joining a saclike vagina (Fig. 8), which is continued as two uteri and ovarian tubules which extend, one anteriorly, the other posteriorly, on opposite sides of the body. The eggs are ovoid, thick-shelled, embryonated, and measure 100 to 160 μ by 30 to 60 μ.

The male tail measures 0.21 to 0.25 mm., is always bent, and tapers sharply to a point (Fig. 9). It is broadest at the cloaca and terminates in a small pointed appendage. There is no sucker present but the preanal musculature is concentrated so as to give the appearance of a depression or groove, running on the ventral side anterior to the anus, to about the level of the first pair of preanal papillae. There are 10 pairs of caudal papillae. Three pairs of mammilate preanal papillae, the posterior pair of which is frequently double, are situated on either rim of the ventral depression. There is a row of three mammilate adanal papillae of about the same size on either side of the anus. There are four pairs of postanal papillae, the anterior pair of which is always mammilate; the other three pairs are sessile and variable in pattern (Figs. 10 and 11). The gubernaculum is triangular, measuring 0.18 to 0.195 mm. long; occasionally it protrudes from the anus with the spicules, the fit being so tight as to create a salience. The spicules are equal and pointed, measuring 1.17 to 1.27 mm.

The only species of *Cruzia* recorded from marsupials is *C. tentaculata* (Rud. 1819) Travassos, 1917 (18) which has been found commonly throughout South and Central America. Other species are recorded from edentates, reptiles, and amphibia. In 1930, Maplestone (16) on the basis of a description by

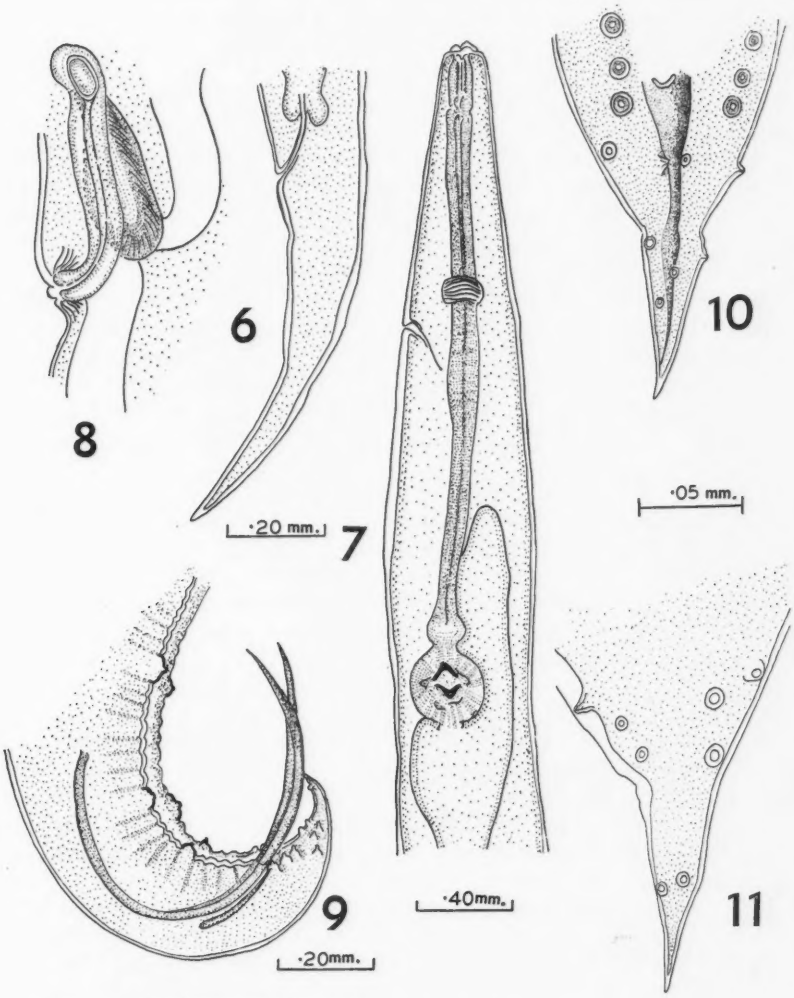


FIG. 6. *Cruzia cameroni*, female tail.
FIG. 7. *C. cameroni*, anterior end of female, showing oesophageal diverticula.
FIG. 8. *C. cameroni*, vulva, ovejector, and sac-like vagina.
FIG. 9. *C. cameroni*, tail of male showing spicules and caudal papillae.
FIG. 10 and FIG. 11. *C. cameroni*, showing arrangement of postanal papillae and variation thereof.

Canavan (8) of a *Cruzia* found by him in *D. marsupialis virginiana*, and following a suggestion by Travassos (18) that the Cruzias of North and South American opossums might be different, named Canavan's form *C. americana*. However, it is impossible to recognize this species because of the insufficiency of Canavan's data and it almost certainly should be considered as a synonym of *C. tentaculata*.

An examination of three species of *Cruzia* shows that the structure of the labia is apparently constant for a given species. At the entrance to the mouth of all three species, on each lip is a pair of papillae, constant in size, character, and arrangement. The stomal opening also appears to be constant for each species. A comparison with *C. testudinis* Harwood, 1932 (13) from the turtle, *Terrapene carolina triunguis*, (specimens of which were generously supplied by Dr. Harwood) (Figs. 12–13), shows these labial characteristics very well. Cephalic structures have been ignored in previous descriptions and this omission renders the separation of species difficult.

My material appears to represent a new species, which is most closely related to *C. tentaculata*. It differs from this species in having the lips separated from the remainder of the body by shoulders, in their angular shape compared with the arched or triangular appearance in *C. tentaculata*, in having amphids raised as appendages to the lips, and in the possession of 10 caudal papillae in the male. The name *C. cameroni* sp. nov. is accordingly proposed for it.

Helminthoxys urichi Cameron and Reesal, 1951 (7)

A single specimen of this oxyurid was found in the stomach of *Didelphis marsupialis insularis* together with several specimens of *Spirocerca*. It is obvious from the location and condition of the worm that it was spurious in this host. The natural location of *H. urichi* is the large intestine of *Dasyproctus agouti*, a rodent common in Trinidad, B.W.I. It was probably ingested with a dead agouti upon which the opossum had fed.

Subulura trinitatis sp. nov.

Four adult worms, three females and one male, were collected from the large intestine of *Philander trinitatis* and a single male from *Didelphis marsupialis insularis*. The specimens from *P. trinitatis* were rather dark and difficult to study because they had been preserved inadequately. The male from *D. marsupialis insularis* however, in the material collected more recently, was light and easily cleared.

These worms are medium to small, 6.1 to 6.9 mm., with a maximum width of 0.39 mm. for the male, and 13.5 to 17.8 mm. by 0.68 mm. for the female. The vestibule is 0.04 mm. long by 0.03 mm. wide and has three teeth at its base (Fig. 18); its walls are cuticularized. The oesophagus measures 1.26 to 1.28 mm. in the male, 1.5 mm. in the female, and terminates in a bulb 0.3 mm. wide. The nerve ring is located about 0.24 mm. and the excretory pore at 0.36 mm. from the anterior end.

In the male, there is a non-cuticularized preanal sucker measuring 0.08 to 0.12 mm. The tail is short and tapering, measuring 0.15 to 0.26 mm. with

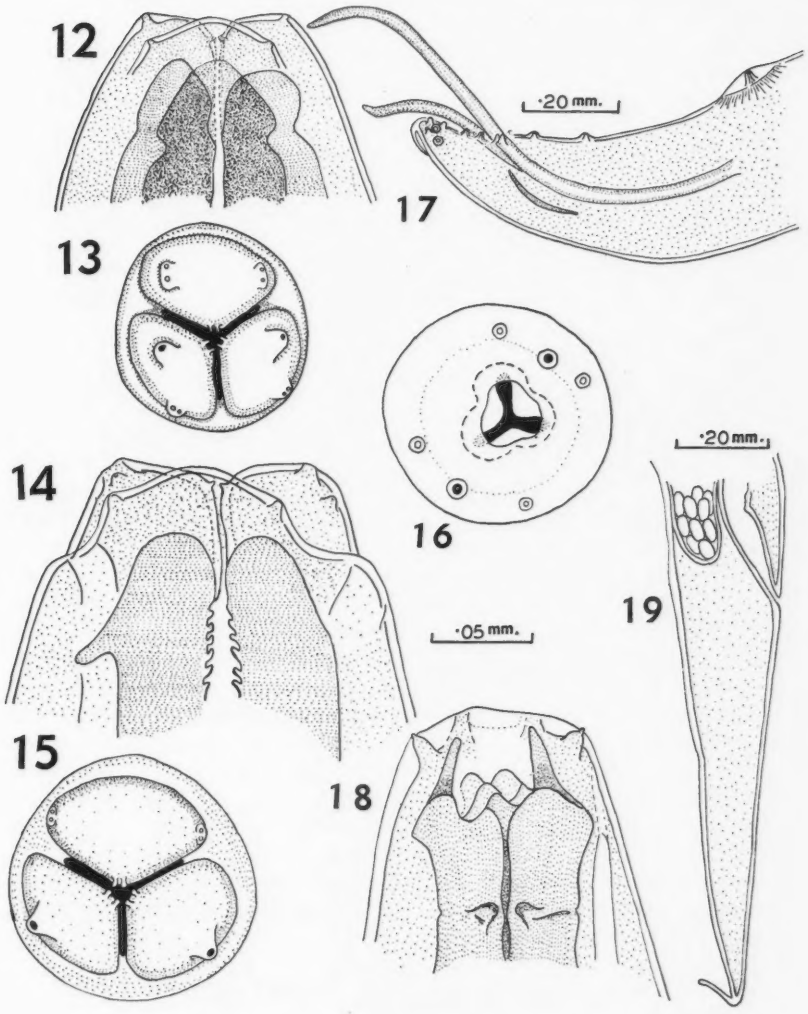


FIG. 12. *C. testudinis* Harwood, 1932, dorsal view of anterior end.
FIG. 13. *En face* view of (12).
FIG. 14. *C. cameroni*, dorsal view, anterior end.
FIG. 15. *En face* view of (14).
FIG. 16. *Subulura trinitatis*, *en face* view showing amphids and papillae.
FIG. 17. *S. trinitatis*, tail of male, spicules, and papillae.
FIG. 18. *S. trinitatis*, lateral view of head of female, showing teeth at base of vestibule.
FIG. 19. *S. trinitatis*, tail of female with spine.

a caudal spine (Fig. 17), measuring 0.11 mm. Caudal alae are present. There are 11 pairs of caudal papillae. Situated at the side of the preanal sucker is the largest preanal papilla. There is also a pair of papillae near the posterior end of the gubernaculum and anterior to the anus. Two pairs of mammilate adanal papillae lie at an angle to the anal opening, the more lateral being the larger. Three of the six pairs of postanal papillae, are situated in a longitudinal–lateral line extending caudad from the anus ending in the caudal alae; the last three pairs of papillae form an inverted triangle in front of the postcaudal spine, the apex being formed by the smallest pair. Spicules are equal and measure 1.28 to 1.58 mm. The gubernaculum, 0.165 mm., is triangular and similar to that described for *S. interrogans* Lent and Freitas, 1935 (15).

Four oral papillae are situated, one on either side of each of two lateral amphids. The mouth is cuticular and round. Slightly below the level of the opening, three teeth are visible at the base of the vestibule (Fig. 16).

The vulva divides the body at the anterior third, is not salient, and leads into a vagina and two divergent uteri. The posterior ovary extends to the level of the anus. Eggs are oval and measure about 68 μ by 45 μ. The female tail is filiform and measures 0.855 to 0.975 mm., with an appendage of 0.08 to 0.11 mm. (Fig. 19).

Three species of *Subulura* have been described from marsupials. *S. peramelis* Baylis, 1930 (2), is the only form from Australian marsupials and is so different that it need not be considered with the South American species, *S. interrogans* Lent and Freitas, 1935 (15) and *S. lanigeri* Foster, 1939 (10). The Trinidad material is regarded as a new species with the name *S. trinitatis* sp. nov. differing from its closest related species, *S. lanigeri* of *Philander laniger pallidus*, in being a smaller worm with smaller spicules and gubernaculum. The vulva of *S. lanigeri* divides the body by 1 : 2, whereas in *S. trinitatis* it divides it by 2 : 3.

Delicata sp.

A single specimen of a female *Delicata* was found in the small intestine of *Didelphis*. As the specimen was in a poor state of preservation, no specific identification could be made.

Fullebornema agoutii (Neiva, Cunha and Travassos, 1915) Travassos and Darriba, 1929

These small, twisted, dark worms were easily recognized by the wide split ramos of the dorsal ray. Ten adults, two males and eight females, were collected from the small intestine of *Didelphis marsupialis insularis*. The natural host is *Dasyproctus agouti* (19) but owing to the location of these worms in the small intestine it is assumed that they were living naturally in *Didelphis*.

Longistriata didelphis (Travassos, 1914) Travassos and Darriba, 1929

Although this is a common trichostrongyle of both North and South American opossums, there were relatively few specimens in this collection.

In some females there is a posterior cuticular sac about the tail. It could not be determined whether the sac was a natural formation or due to loosening of the cuticle in fixing. If it is natural, it differs from the character of the female tail usually described (19).

Viannaia hamata Travassos, 1914

Specimens of this were identified from *D. marsupialis insularis* although a large ventral ala, which has not previously been described for this genus, was conspicuous in all; it extends the length of the worm (19). This species has been previously recorded from *D. aurita* in Brazil (9).

Camerostrongylus didelphis gen. et sp. nov.

Several specimens of this trichostrongyle were collected from the small intestine of *Didelphis marsupialis insularis*. The females measure 5.00 to 5.31 mm. long by 0.08 mm. wide, the males 3.66 to 3.93 mm. long and 0.08 mm. wide. Transverse and longitudinal lines are present and raised to form alaeform expansions. These are widest at midbody and become narrower at the cephalic end (Fig. 20), and look like saw teeth in lateral view, owing to the wavy character which is seen from end-on-views. There are about eight of these expansions which converge as they approach the cephalic cuticular expansion (Fig. 21).

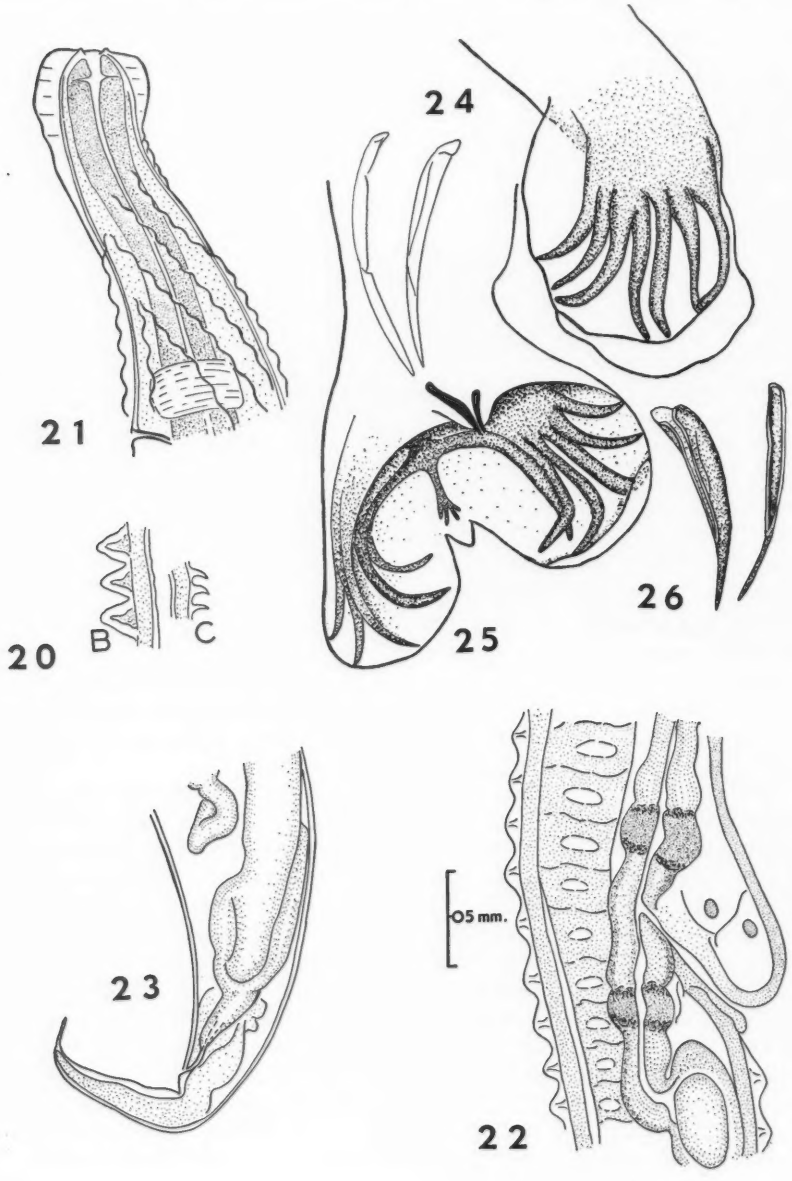
The cephalic end has a dorsal bend. The mouth opening is simple and surrounded by six circumoral papillae. No buccal cavity is present. The oesophagus is club-shaped and simple, measuring 0.195 to 0.345 mm. in the males and 0.390 to 0.410 mm. in the females. The nerve ring is 0.2 mm. from the anterior end with the simple excretory pore at the same level. Cervical papillae are absent.

The vulva is covered by a cuticular flap (Fig. 22) and is located at the beginning of the posterior fifth of the body. There are two *pars ejectrix* which accept eggs from anterior and posterior uteri. Eggs are oval, thin shelled, and nonembryonated, measuring 30 by 40 μ.

The tail (Fig. 23) is pointed, conical, and terminates in a fine point. It measures 0.105 to 0.165 mm. The intestine in the female ends in a conspicuous rectum which empties by a thin tube through the anus.

The bursa of the male has two large, asymmetrical, lateral lobes and a small dorsal lobe (Figs. 24, 25). The ventral and lateral rays are completely separated but appear to have a common trunk. All bend to the cephalic end and are about the same size. The anterolateral ray is large, running parallel to the ventrals and reaching the bursal margin. The mediolateral and posterolateral extend with the anterolateral for most of their length, but turn posterior in the distal fourth, reaching the bursal margin. The externodorsal is as large as the laterals; branching from the base of the dorsal trunk, it extends parallel to, and at some distance from, the laterals, ending in the bursal membrane. The dorsal is short, splitting into two forks at its distal end. There are small prebursal papillae at the junction of the bursa and the body.

The spicules are short, equal, and simple, measuring 0.110 to 0.153 mm. (Fig. 26). Their proximal ends are broad, the distal pointed, but not filamentous. A triangular, almost transparent gubernaculum, measuring 0.08



to 0.09 mm. is present, lying in the genital cone which protrudes into the bursal cavity.

Camerostrongylus resembles most closely *Citellinema* Hall, 1916, and is placed accordingly in the subfamily Ornithostrongylinae Travassos, 1937. It differs from *Citellinema* in that the anterolateral ray reaches the bursal margin and the spicules are simple. A gubernaculum is present and the cuticular characters differ. *Macielia* Travassos, 1936 (19), a genus from marsupials, is also similar but has a long dorsal and short externodorsal ray, as well as different types of spicules and excretory pore.

Philostrongylus philanderi gen. et sp. nov.

These trichostrongyles were taken from the large intestine of *Philander trinitatis*. They are small, slender, threadlike worms which are twisted in appearance, but not coiled into spirals. Females are more than twice as long as the males, measuring 12.36 to 13.92 mm. The males measure 5.48 to 5.54 mm. with a maximum width of 0.15 mm. There is a cephalic expansion of 0.040 mm. by 0.039 mm. bearing transverse lines (Fig. 27). There is a circle of circumoral papillae and the mouth is simple. The buccal capsule is simple and short but not sclerotic. The oesophagus measures 0.34 to 0.41 mm. and has an expansion at the level of the intestinal junction. The nerve ring is located at 0.26 mm. in the female and 0.14 mm. in the male from the anterior end, with the excretory pore at the same level.

The vulva is terminal (Fig. 28) and opens next to the anus, connecting with the complex ovejector. There are two vulvular sphincters, the anterior one ending in a valve apparatus of six flaps which open and close to permit the eggs to move outward only (Fig. 29). A posteriorly located atrium receives the eggs, has a heavy, muscular wall, and is cone-shaped; all its fibers run toward the vulva and the walls are folded. This atrium opens through the vulva to the outside. A cuticular sac covers the caudal end. The tail in all specimens was blunt, without cone or point. The uterus contains about 50 eggs in single file, which are in the two-cell stage when laid. They measure 66 μ by 33 μ at the entrance to the ovejector.

The bursa is divided into two equal lobes (Figs. 31, 32). There is a slit in the margin caudad to the dorsal ray. The bursal formula is as follows: lateral and ventral rays arise from separate stems. The ventroventral extends toward the bursal margin for half its length and bends sharply cephalad, reaching the anterior margin; the lateroventral extends to the margin. The anterolateral, after a short curve near its tip, ends in the form of a protruding

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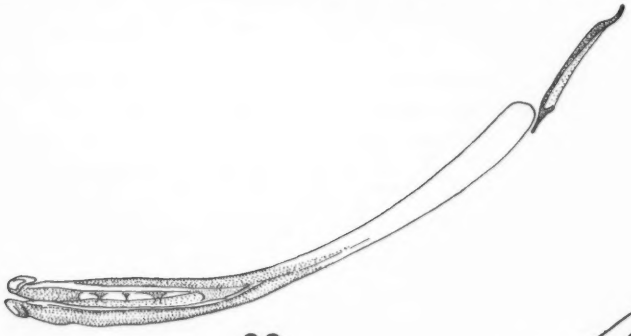
FIG. 20. *Camerostrongylus didelphis*, lateral view of linear cuticular expansions—(B) near vulva, (C) near head.
FIG. 21. *C. didelphis*, head of female showing fusion of expansions with body cuticle.
FIG. 22. *C. didelphis*, vulva.
FIG. 23. *C. didelphis*, tail end showing rectal swelling.
FIG. 24. *C. didelphis*, lateral view of bursa.
FIG. 25. *C. didelphis*, ventral view of bursa.
FIG. 26. *C. didelphis*, spicules.



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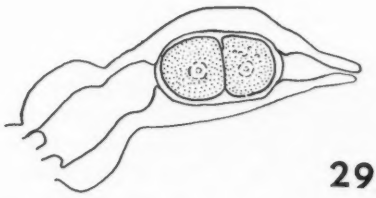

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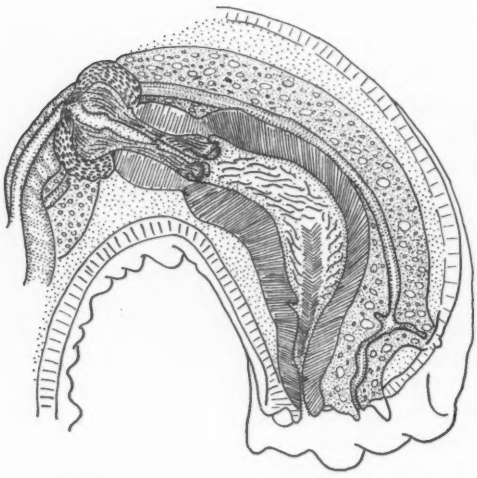

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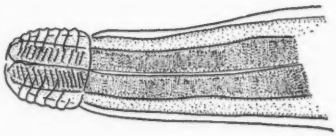

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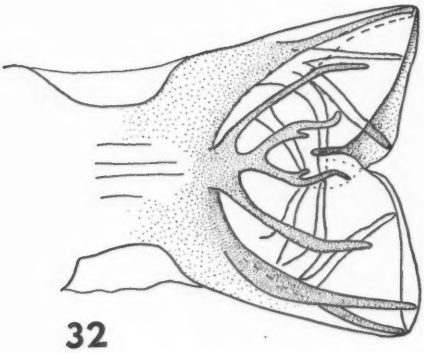

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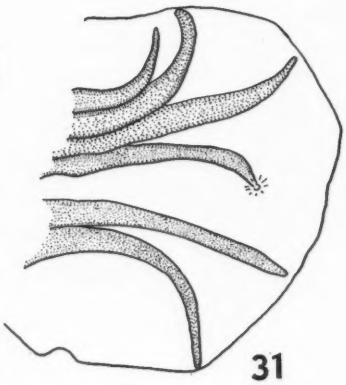

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papilla about a quarter of its length from the bursal margin. The mediolateral extends straight out, ending in or near the bursal margin. The posterolateral curves caudal in its posterior third, ending in the bursal margin alongside the externodorsal. The dorsals arise from a common trunk. Externodorsals are thin and delicate, splitting off near the base and extending outward at about 45° from the dorsal and ending in the membrane. The dorsal ray forks above the externodorsal giving rise to a long branch extending caudad and two short internal branches.

The spicules are long and thin distally, the proximal end being stockier and more complex (Fig. 30). There seems to be a fusion of the spicules caudal to the broader proximal part. They measure 0.303 to 0.306 mm. A gubernaculum is present and measures 0.05 to 0.06 mm. A slight genital cone at the caudal end protrudes into the bursal cavity.

This genus belongs to the subfamily Viannaiinae Neveu-Lemaire, 1934, resembling *Longistriata* more closely than any of the other recorded genera. It differs in not having a conical tail in the female; both the vulva and the anus are side by side terminally. *Avellaria* Freitas and Lent, 1934, (12) has a tail characterized as being similar to *Philostrongylus*, but even in this genus there is a slight conical tip, and the vulva is situated farther from the anus. Other characters of *Avellaria* are different.

Foster (10) remarks on the lack of trichostrongyles from the genus *Philander* and records one species, *Macielia macieli* (Travassos, 1915) Travassos, 1935, from *P. laniger pallidus*.

Spirocerca cylicola sp. nov.

Specimens of *Spirocerca* were collected from the stomach and small intestine of three specimens of *Didelphis marsupialis insularis*. Intestinal forms of this worm were smaller than those found in the stomach. Only one male and eight females were available. All were adult.

Females from the stomach measure 8.0 to 8.5 mm. in length, those from the intestine 4.7 to 5.7 mm. The single male was 1.52 mm. long by 0.15 mm. wide. The mouth is circular, 0.08 mm. wide. It leads into the vestibule which is not surrounded by teeth as usually described for *Spirocerca* but by extensions of the sclerotic vestibular wall (Fig. 33). The vestibule is 0.06 to 0.07 mm. long by 0.015 mm. wide. The oesophagus is long (0.80 to 1.47 mm.) and divided into two portions which bear an average ratio of about 1 : 4. The anterior muscular portion is about 0.15 mm. and a long posterior end about 0.75 mm. The nerve ring and excretory pore lie at about 0.21 to 0.26 mm. from the anterior end of the worm.

FIG. 27. *Philostrongylus philanderi*, female, anterior end of cuticular expansion.
FIG. 28. *P. philanderi*, female, posterior end showing detail of vulva.
FIG. 29. *P. philanderi*, character of the ovejector with detail of egg.
FIG. 30. *P. philanderi*, spicules and gubernaculum.
FIG. 31. *P. philanderi*, lateral view of bursa.
FIG. 32. *P. philanderi*, dorsal view of bursa.

The female tail is short and stumpy, bearing several ornamental points upon its tip and measures 0.10 to 0.15 mm. in length (Fig. 34). The intestine ends in the form of a rectal swelling which is variable in size.

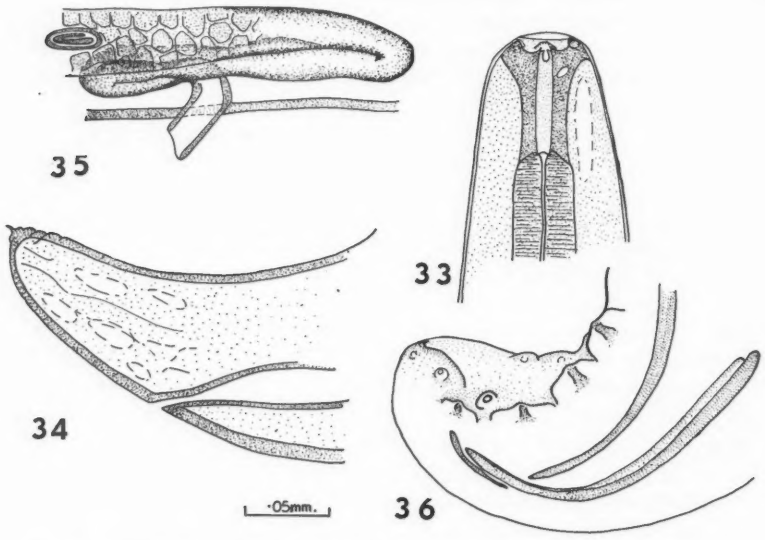


FIG. 33. *Spirocerca cylicola*, lateral view of the female head showing vestibule.
FIG. 34. *S. cylicola*, tail of female showing posterior tip.
FIG. 35. *S. cylicola*, vulva and ovejector of female.
FIG. 36. Tail of male.

The vulva, located about the middle of the body, is salient (Fig. 35), resembles a sclerotized tube and extends beyond the body wall at an angle of about 45°. There is a vagina which folds against itself before leading into the amphidelphis uteri, which, when filled, obscures all other organs. The anterior ovary reaches to the level of the junction between muscular and glandular oesophagus. The posterior one reaches nearly to the anus.

The eggs, massed one upon the other in the uterus, are elongate, embryonated when laid, and measure about 26 μ by 10 μ.

The spicules are unequal, the left being the longer and thinner, 0.42 mm., and the right the shorter and heavier, 0.22 mm. The gubernaculum measures 0.06 mm.

The male tail is coiled several times. It is short, alate, and measures 0.05 mm. Two lateral lobes of the caudal alae bear four large pairs of preanal papillae (Fig. 36) and one large unpaired papilla on the median line. At the posterior tip, which is rounded, are two pairs of prominent postanal papillae. Other papillae may have been present but were not observable owing to the state of preservation. There were no cervical papillae.

Hill (14) describes *S. longispiculata*, from the stomach of *Didelphis marsupialis virginiana* in the United States. The Trinidad form is similar to it but differs in the ratio of muscular to glandular parts of the oesophagus, the absence of cervical papillae, the presence of a gubernaculum, and a longer female tail (about twice as long as that of *S. longispiculata* according to Hill's figure). The character of the vulva is not discussed by Hill.

The only other species of *Spirocerca* reported from marsupials is *S. heydoni* Baylis, 1927 (1) from Australia. This species differs from either of the aforementioned by having a longer tail, vulva anterior, and stomal teeth.

Accordingly, the Trinidad forms are regarded as belonging to a new species for which the name *S. cylicola* sp. nov. is proposed.

Physaloptera turgida (Rud., 1819)

Two specimens were collected from the stomach and intestine of *Philander trinitatis* and three from *Didelphis marsupialis insularis*. This worm appears to be peculiarly scarce in Trinidad marsupials considering that it is one of the commonest helminths of South American opossums. Another odd point is that specimens from the intestine were much larger than those from the stomach; in the North American opossum, *Didelphis marsupialis virginiana*, the reverse situation exists.

Cortasomoides philanderi Foster, 1939

Nine adult worms, four males and five females, were collected from the body cavity of *Philander trinitatis*. The females were slightly larger than the average recorded by Foster (10) and the vulva farther cephalad. The tail is 60 μ long. Other characters are the same as those recorded by Foster.

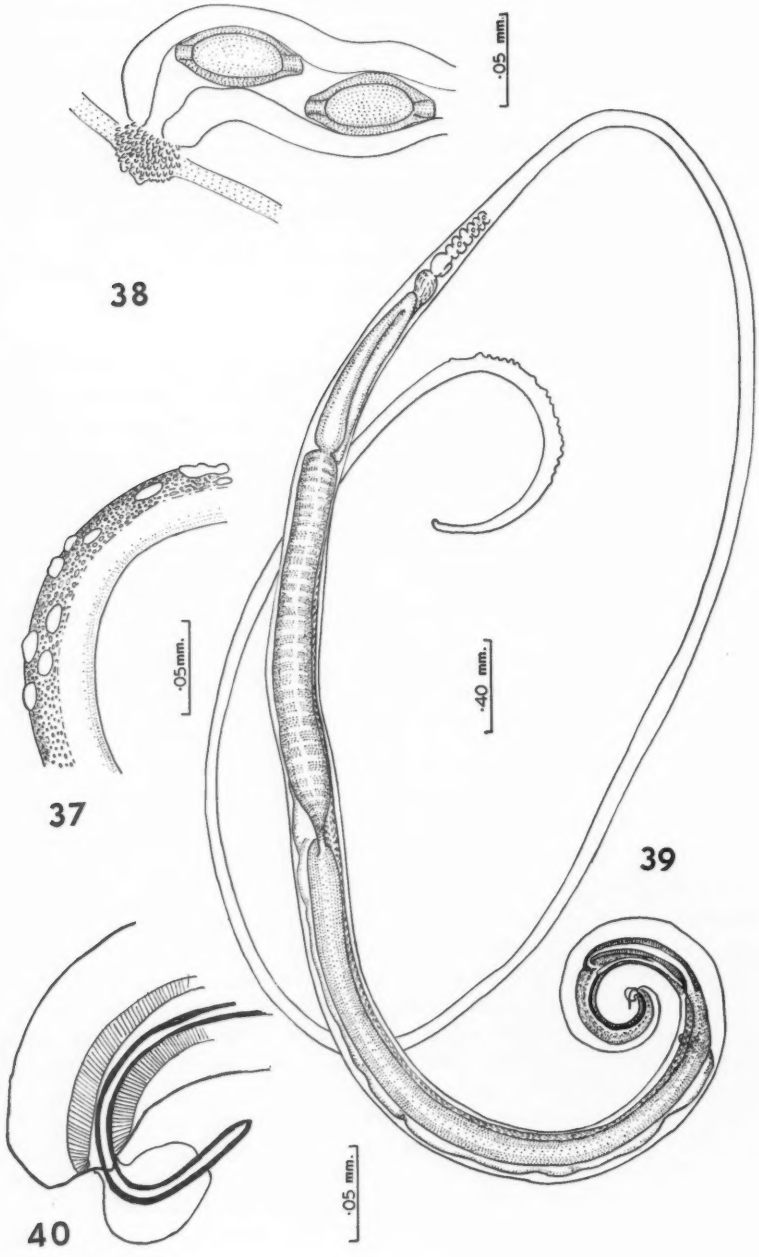
Trichuris minuta (Rud., 1819)

Several specimens were taken from the intestine of *Didelphis marsupialis insularis*. They were in poor condition but the size of the body (about twice the body lengths recorded for the other two species of *Trichuris*) and the character of the "neck" suggest that they belong to this species.

Trichuris urichi sp. nov.

Several specimens were taken from the large intestine of *Didelphis marsupialis insularis*. The female body is 2.93 to 4.78 mm. long by 0.32 to 0.41 mm. wide. The neck is 9.05 to 10.00 mm. long, or at least three times as long as the body. A salient vulva surrounded by many papillaform projections (Fig. 38) gives the effect ventrally of a composite flower. The bacillary band reaches from the anterior end to about the level of the junction of neck to body. It has intermittent blisterlike protuberances (Fig. 37).

The ovejector and vagina are muscular and together measure 0.375 to 0.645 mm. Eggs are tandem in arrangement and few in number in the vagina. The ovary extends from the posterior end of the body anteriorly, then curves at the level of the vagina posteriorly. At the posterior end it broadens into the gravid uterus. The eggs have definite protruding plugs and measure 66 μ by 30 μ (Fig. 38).


.05 mm.
38
.05mm.
37
.40 mm.
39
.05 mm.
40

The body of the male is elongate, measuring 3.93 to 6.08 mm. and slightly thicker than the neck, which measures 8.17 to 9.44 mm., or about one and one-quarter to twice as long as the body (Fig. 39).

The vas deferens is from 0.80 to 1.64 mm. long. It constricts posteriorly forming the ejaculatory duct (1.45 to 2.55 mm. in length) which joins the intestine, forming a cloacal tube, 0.53 to 0.66 mm. long. After joining the spicule tube, the cloacal tube extends 0.39 mm. to the anus. The spicule is filiform and measures 0.75 to 0.86 mm. long. The sheath is campanulate and the tail is coiled (Fig. 40).

This species is regarded as new for the reasons noted below (Table II) and the name *T. urichi* sp. nov. is proposed for it.

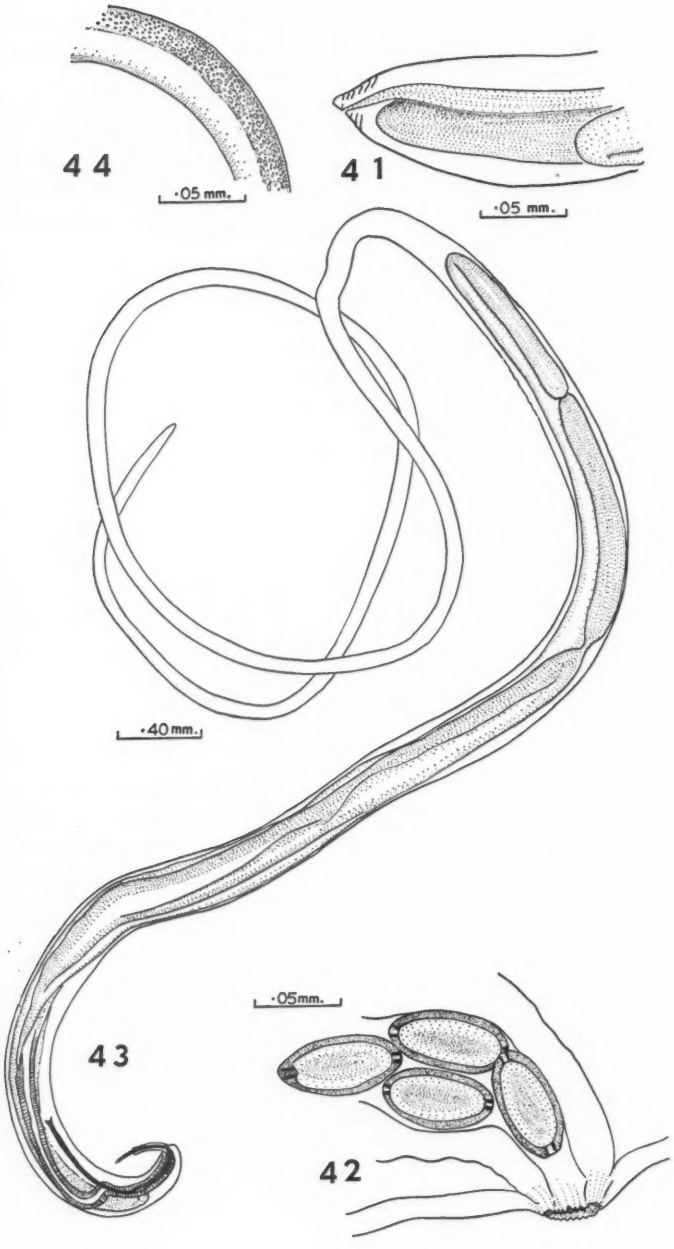
TABLE II

	T. marsupialis, mm.	*T. urichi*, mm.	*T. reesali*, mm.
Vas deferens	1.40	0.8 – 1.6	0.9 – 1.6
Ejaculatory duct	1.58	1.5 – 2.6	1.8 – 2.5
Cloacal tube	0.86	1.53 – 0.66	1.4
Spicule tube	0.12	0.68 – 0.84	1.4 – 1.9
Spicule	0.73	0.75 – 0.86	0.9 – 1.08
Vulva	Salient, smooth	Papillate, salient	Salient, smooth
Vagina	0.67	0.38 – 0.65	1.02 – 1.25
Sheath	Bell	Bell	Tube, tight-fitting
Tail	Coiled	Coiled	Straight
Bacillary band	—	Blisters or protuberances	No blisters

Trichuris reesali sp. nov.

There were found in *Didelphis* some specimens of *Trichuris* which upon first examination appeared to be *Trichuris urichi*. Further study of the genitalia in the male and of the egg and vulva characters in the female, indicated that they were a new and different species. The female body measured 4.4 mm. to 4.8 mm. long, the neck, 10.64 mm. in one specimen; in another specimen, in which the neck was broken, it was probably even longer. The head is simple with no alae. There is at the posterior tip a retractile papilla similar to that described by Baylis (3). The vulva is at the junction of the neck and body and has a definite rim which is unornamented and slightly salient (Fig. 42). The vagina is elongate, muscular, and contains several eggs. There are usually four eggs grouped in the ovejector at the vulva suggesting that they may be laid in that number. The combined vagina – ovejector measures 1.02 to 1.25 mm., broadening into the uterus at the posterior end.

FIG. 37. *Trichuris urichi*, bacillary band.
FIG. 38. *T. urichi*, vulva with eggs.
FIG. 39. *T. urichi*, adult male, showing relationship of genital tubules.
FIG. 40. *T. urichi*, tail of male.


44
·05 mm.
41
·05 mm.
·40 mm.
43
·05mm.
42

The ovary begins at the posterior end as it does in *Trichuris urichi* and turns posterior within 0.53 to 0.57 mm. of the vulva. The eggs are nearly oval, the plugs not protruding, and measure 65 μ by 30 μ.

In the male the neck is less than twice the length of the body (Fig. 43): the oesophageal region is 8.12 to 9.93 mm. and the posterior end 4.25 to 6.17 mm. long, with a width similar to the ejaculatory duct which measures 1.80 to 2.48 mm. The cloacal tube is thick-walled, 1.35 to 1.44 mm. long. The spicule tube appears to be long and in some specimens bent almost around the worm in a spiral, a feature which renders measurement difficult; its length varies between 1.35 and 1.91 mm. The spicule is filiform, measuring 0.90 to 1.08 mm. The sheath, excerted in two specimens and retracted in two, measures 0.15 mm. and bears several rows of spines which spiral toward the body, fitting tightly about the spicule.

There is a bacillary band on the neck which reaches from the anterior end to about the level of the junction of the oesophagus and intestine. There are no blisters or protuberances (Fig. 44).

T. reesali and *T. urichi* are most closely related to *T. marsupialis* Foster, 1939, but both differ from the latter species in several important respects. Table II gives the characters in which they differ.

T. reesali differs from *T. marsupialis* in the character of the vulva; the ejaculatory duct is at least twice as long as the vas deferens, and the spicule tube is longer. The spicules and the vagina in *T. reesali* are significantly longer.

T. reesali differs from *T. urichi* in having a longer cloacal tube, a different type of vulva, longer spicules and vagina, longer spicule tube, different egg characteristics, a straight tail, tight-fitting spicule sheath, a longer cloacal tube in proportion to other genitalia, and a different type of bacillary band.

Capillaria sp.

One incomplete specimen of a female capillarid was found in *Didelphis marsupialis insularis*. The tail had been broken off, presumably during removal from the host, but the head end and a large part of the body were still intact.

The worm was collected from the small intestine and measures 53.55 mm. to the break. The oesophagus is 11.12 mm. long and the head is simple as shown in the figure (Fig. 46). The vulva is 11.18 mm. from the cephalic end and is simple (Fig. 45). The vagina is long, measuring 2.75 mm., and the eggs are grouped in a single file. The eggs measure 0.255–0.240 mm. long, with the plugs 0.195 to 0.202 mm. long by 0.12 to 0.11 mm. The uteri are voluminous and contain large numbers of eggs.

FIG. 41. *T. reesali*, tail of female.
FIG. 42. *T. reesali*, vulva of female.
FIG. 43. *T. reesali*, adult male showing relation of genital tubules.
FIG. 44. *T. reesali*, bacillary band.

Of the Capillarias referred to in the literature, only *Capillaria fluminensis* Freitas, 1946 (11) comes close to the length of the specimen under study. It measures 69.88 mm. for the female. However, the vagina is shorter and the eggs are smaller than these specimens.

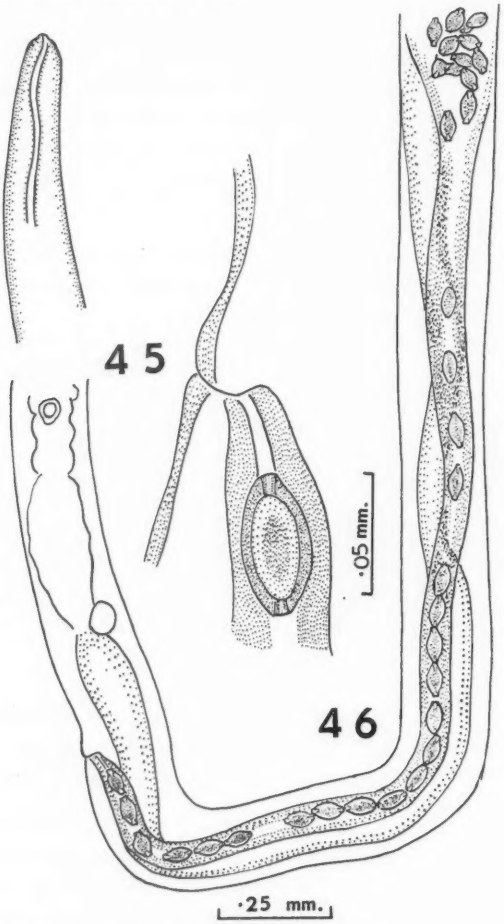


FIG. 45. *Capillaria* sp., vulva and eggs.
FIG. 46. *Capillaria* sp., beginning of oesophagus.

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